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(54) Title: A METHOD OF REGULATION

(57) Abstract

The present invention relates generally to a method of regulating interferon functional activity and agents used for the same. More particularly, the present invention contemplates a method of regulating the functional activity of type I interferon by administering a soluble type I interferon receptor, and still more particularly IFNAR 2a. The method of the present invention is useful, *inter alia*, in a range of therapeutic and prophylactic applications.

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A METHOD OF REGULATION

FIELD OF THE INVENTION

The present invention relates generally to a method of regulating interferon functional activity and agents used for the same. More particularly, the present invention contemplates a method of regulating the functional activity of type I interferon by administering a soluble type I interferon receptor, and still more particularly IFNAR 2a. The method of the present invention is useful, *inter alia*, in a range of therapeutic and prophylactic applications.

BACKGROUND OF THE INVENTION

The bibliographic details of the publications referred to by author in this specification are collected at the end of the description.

The type I interferons are pleiotropic cytokines which, in all vertebrate species, can impart important signals to cells to protect against viral infection, inhibit proliferation, and activate immune effector cells (Pestka *et al.*, 1987). They have also been shown to play a role in inhibiting tumour cell growth. However, in addition to their beneficial effects, interferons also induce adverse side effects such as the inhibition of normal cell growth, undesirable modulation of the functional activity of other cytokines or other unwanted immune effects such as fever, malaise, nausea or leukopenia. These unwanted effects are often evidenced as the unwanted side effects of interferon therapy. Interferons have also been associated with the development and/or progression of autoimmune disease and possibly Aicardi-Goutières Syndrome.

The human type I interferons include multiple subtypes of interferon-α, a single interferon-β, and in some species interferon-ϖ and -τ (Weissman *et al.*, 1986; Roberts *et al.*, 1991). The structure of type I interferons is highly conserved, ranging from 70-98%

amino acid identity between interferon-α subtypes to 35% identity between interferon-α and interferon-β (Weissman *et al.*, 1986). Not only are type I interferons structurally and functionally related, but they also compete with each other for receptor binding and therefore share one or more common receptor component(s) (Uzé *et al.*, 1995).

Two components of the human type I interferon receptor have been cloned. The first component of the type I interferon receptor to be cloned was human IFNAR 1 (Uzé *et al.*, 1990). The cDNA encoding the second component was cloned (Novick *et al.*, 1994) and is designated IFNAR 2. Studies have identified that the human IFNAR 2 gene encodes multiple mRNA transcripts which are translated into several isoforms: a soluble form designated as IFNAR 2a (Novick *et al.*, 1994; Lutfalla *et al.*, 1995), a "short" transmembrane form designated as huIFNAR 2b (Novick *et al.*, 1994; Lutfalla *et al.*, 1995) and a "long" transmembrane form designated as huIFNAR 2c (Lutfalla *et al.*, 1995; Domanski *et al.*, 1995). The reason for the existence of two transmembrane isoforms of IFNAR 2 is unknown.

The role and contribution of IFNAR 1 and IFNAR 2, either separately or together, to ligand binding and signal transduction remains unknown. In fact, various *in vitro* functional analyses of transmembrane IFNAR 1 and 2 have produced contradictory results. With respect to soluble IFNAR 2a, there is little known of the expression of this receptor, such as whether it is found *in vivo* as a circulating protein or whether it exhibits any biological activity.

Understanding the mechanisms by which cytokines mediate their functional activity is necessary if methods of regulating cytokine functional activity for therapeutic or prophylactic application are to be developed. For example, it is necessary to elucidate the role and mechanism of action of molecules which bind cytokines.

In work leading up to the present invention the inventors have mapped the expression of the IFNAR 2a molecule and have surprisingly found that expression of this molecule is regulated independently of the transmembrane receptor, it is found in high levels in

biological fluids and exhibits biological activities. Soluble IFNAR 2a has been found to inhibit the functional activity of type I interferon. However, even more surprisingly, in some circumstances IFNAR 2a acts to present a type I interferon ligand to the IFNAR 1 receptor chain and facilitates the transduction of a signal into the cell. This has led to the development of a method of regulating type I interferon functional activity utilising the soluble IFNAR 2a molecule or its derivatives thereof.

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step of group of integers or steps.

One aspect of the present invention provides a method of regulating, in a subject, interferon functional activity said method comprising administering to said subject an effective amount of a soluble interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said soluble interferon receptor to couple, bind or otherwise associate with said interferon.

Yet another aspect of the present invention provides a method of regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said soluble type I interferon receptor to couple, bind or otherwise associate with said type I interferon.

Still another aspect of the present invention provides a method of regulating, in a subject, interferon α and/or interferon β functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative

thereof for a time and under conditions sufficient for said soluble type I interferon receptor to couple, bind or otherwise associate with said interferon α and/or interferon β.

Still yet another aspect the present invention is directed to a method of regulating, in a subject, interferon functional activity said method comprising administering to said subject an effective amount of a soluble interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof coupled, bound or otherwise associated with type I interferon, or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said complex to regulate interferon functional activity.

Still another aspect of the present invention provides a method of regulating, in a subject, interferon α and/or interferon β functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative thereof for a time and under conditions sufficient for said soluble type I interferon receptor to couple, bind or otherwise associate with said interferon α and/or interferon β.

Another aspect of the present invention is directed to a method of regulating, in a subject, interferon functional activity said method comprising administering to said subject an effective amount of a soluble interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof coupled, bound or otherwise associated with type I interferon, or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said complex to regulate interferon functional activity.

A further aspect of the present invention provides a method of down-regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said receptor to couple, bind or otherwise associate with said type I interferon.

Another further aspect of the present invention is also directed to a method of up regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said receptor to couple, bind or otherwise associate with said type I interferon.

Yet another further aspect of the present invetion provides a method of up-regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative, homologue, analogue, chemical equivalent or mimetic thereof coupled, bound or otherwise associated with type I interferon or derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to up-regulate type I interferon functional activity.

Another aspect of the present invention provides a method of regulating type I interferon functional activity in a subject said method comprising administering to said subject an effective amount of a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for the expression product of said nucleic acid molecule to couple, bind or otherwise associate with said type I interferon.

Still another aspect of the present invention provides a method of regulating, in a subject, type I interferon functional activity said method comprising contacting *IFNAR 2* with an effective amount of an agent for a time and under conditions sufficient to up-regulate, down-regulate, or otherwise modulate expression of *IFNAR 2*.

Another aspect of the present invention contemplates a method of regulating activity of type I interferon in a subject, said method comprising administering to said subject an effective amount of an agent for a time and under conditions sufficient to modulate IFNAR 2a activity.

Yet another aspect of the present invention provides a method for the treatment or prophylaxis of a disease condition characterised by unwanted type I interferon functional activity in a subject said method comprising administering to said subject an effective amount of one of more of:

(i) a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(ii) a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, equivalent, homologue, analogue or mimetic thereof;

(iii) an agent capable of modulating the activity of a soluble type I interferon receptor; or

(iv) an agent capable of modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor

sufficient to down-regulate the functional activity of said type I interferon.

A further aspect of the present invention there is provided a method for the treatment or prophylaxis of a disease condition characterised by an inadequate type I interferon response in a subject said method comprising administering to a subject an effective amount of one or more of:

(i) a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(ii) a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, equivalent, homologue, analogue or mimetic thereof;

(iii) an agent capable of modulating the activity of a soluble type I interferon receptor; or

(iv) an agent capable of modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor

sufficient to up-regulate the functional activity of said type I interferon.

Still yet another aspect of the present invention provides an agent useful for regulating interferon functional activity comprising a soluble interferon receptor, nucleic acid molecules encoding said receptors or agents which regulate the activity or expression of said receptors or encoding nucleic acid molecules as hereinbefore defined.

Another aspect of the present invention relates to a method of treating a subject said method comprising administering to said subject an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *IFNAR 2* or sufficient to modulate the activity of IFNAR 2a wherein said modulation results in modulation of type I interferon functional activity.

In another aspect the present invention relates to a method of treating a subject said method comprising administering to said subject an effective amount of IFNAR 2a or *IFNAR 2* or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient to regulate type I interferon functional activity.

Yet another aspect of the present invention relates to the use of an agent capable of modulating the expression of *IFNAR 2* or modulating the activity of IFNAR 2a in the manufacture of a medicament for the regulation of type I functional activity.

A further aspect of the present invention relates to the use of IFNAR 2a or *IFNAR 2* or derivative, homologue, analogue, equivalent or mimetic thereof in the manufacture of a medicament for the regulation of type I interferon functional activity.

Still yet another aspect of the present invention relates to agents for use in modulating *IFNAR 2* expression or IFNAR 2a activity wherein said regulation results in modulation of type I interferon functional activity.

Another aspect of the present invention relates to IFNAR 2a or *IFNAR 2* or derivative, homologue, analogue, equivalent or mimetic thereof for use in modulating type I interferon functional activity.

A further aspect of the present invention provides a pharmaceutical composition for use in regulating interferon functional activity comprising a soluble interferon receptor as hereinbefore defined, *IFNAR 2* or derivative, homologue, analogue, equivalent or mimetic thereof or an agent capable of modulating *IFNAR 2* expression or IFNAR 2a activity together with any one or more pharmaceutically acceptable carriers and/or diluents.

Still another further aspect of the present invention relates to a method of determining type I interferon non-responsiveness in a subject said method comprising screening for the level of a soluble type I interferon receptor or a derivative, homologue, analogue, equivalent or mimetic thereof in a body fluid of said subject wherein the level of said soluble receptor or derivative, homologue, analogue, equivalent or mimetic thereof relative to the normal level of said soluble receptor or derivative, homologue, analogue, equivalent or mimetic thereof is indicative of type I interferon non-responsiveness.

Another aspect of the present invention provides a kit for detecting soluble type I interferon receptor said kit comprising in compartmental form a first compartment adapted to contain a type I receptor specific immunointeractive molecule and a second compartment adapted to contain reagents useful for visualising said immunointeractive molecule.

Yet another aspect of the present invention is directed to antibodies to soluble type I interferon receptor molecules. Such antibodies may be monoclonal or polyclonal and may

be selected from naturally occurring antibodies or may be specifically raised to said molecules. The present invention extends to recombinant and synthetic antibodies and to antibody hybrids.

Another further aspect of the present invention provides a method for detecting an agent capable of modulating the functional activity of type I interferon, said method comprising contacting a cell or extract thereof, which cell or extract thereof contains IFNAR 1 and/or IFNAR 2, or its functional equivalent or derivative thereof, with an IFNAR 2a-interferon complex and a putative agent and detecting an altered expression phenotype associated with said type I interferon.

In another aspect, the present invention should also be understood to extend to secondary screening methods which are designed to detect agents which regulate the activity of interferon induced signals, which screening methods do not require the use of IFNAR 2a and/or type I interferon during the testing procedure.

In still another aspect, the present invention relates to modulatory agents detected in accordance with the screening method herein disclosed.

Still another aspect of the present invention relates to the use of said modulatory agents to modulate type I interferon functional activity in accordance with the method of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a photographic representation of **A**, Northern blot analysis of poly(A)$^+$ mRNA (3μg) from mouse organs and cell lines probed with full-length murine IFNAR-2 cDNA. Filters were stripped and then re-hybridised with a probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). **B**, Ratios of the soluble to transmembrane muIFNAR-2 mRNA transcripts from Northern blot analysis. A phosphorimage analyser was used to quantitate transcript intensities. Data is presented as the mean ±SEM of replicate experiments.

Figure 2 is a photographic representation of **A**, Northern blot of poly(A)$^+$ mRNA from whole mouse embryos and embryonic organs at different stages of development, probed with full-length murine IFNAR-2 cDNA then *GAPDH* as a control. **B**, Ratios of the soluble to transmembrane muIFNAR-2 mRNA transcripts in murine embryonic, neonatal and adult tissues following Northern blotting. A phosphorimage analyser was used to quantitate transcript intensities.

Figure 3 is a photographic representation of the Western blot analysis of soluble muIFNAR-2a expression. Western blot analysis of **A**, Recombinant muIFNAR-2a (*E.coli*)(lane 1), muIFNAR-2 ECD (*P.pastoris*)(lane 2) and conditioned media from COS7 cells transiently transfected with muIFNAR-2a cDNA (lane 3), muIFNAR-2c cDNA (lane 4) and pEF-BOS (plasmid control) (lane 5) **B**, Competition with recombinant muIFNAR-2a in Western blots of conditioned media from COS7 cells transiently transfected with muIFNAR-2a cDNA. The amounts of muIFNAR-2a used are as indicated, and were pre-incubated with the competing antibody before being added to the blot.

Figure 4 is a photographic representation of the Western blot analysis of soluble muIFNAR-2a in murine biological fluids. Western blot analysis of **A**, Serum from mice normal (+/+) and homozygous (-/-) for a null mutation of the *muIFNAR-2* gene. **B**, Recombinant muIFNAR-2a competition to demonstrate specificity of soluble receptor detection in normal murine serum. **C**, Western blots of soluble receptor in urine (lane 1), peritoneal fluid diluted 1:2 (lane 2), serum diluted 1:20 (lane 3) and murine saliva (lane

4). D, Western blots of soluble receptor in urine (lane 1), 3T3 cell-line conditioned media (lane 2) and L cell conditioned media (lane 3).

Figure 5 is a graphical representation of the Inhibition of type I IFN-induced gene expression in murine L929 cells by soluble muIFNAR-2a. Measurement of fold induction of the activity of luciferase driven by a 2'-5' OAS promoter following incubation of transfected L cells with A, 10IU/ml (■), 1IU/ml (▲) and 0 recombinant muIFNα1 (▼) or B, 10IU/ml (■), 1IU/ml (▲) and no recombinant muIFNβ (▼), with the indicated concentrations of muIFNAR-2a. The data is presented as the mean ±SEM (n = 8) fold induction of luciferase activity *versus* concentration (ng/ml) muIFNAR-2a used.

Figure 6 is a graphical representation of the Inhibition of the antiproliferative effect of IFNα on primary thymocytes by soluble muIFNAR-2a. Thymocytes stimulated to proliferate with PHA (40μg/ml) were incubated with A, 100IU/ml (▽) 10IU/ml (▲) recombinant muIFNα4 or B, no muIFNα4, with the indicated concentrations of recombinant muIFNAR-2a. Their DNA proliferation was measured by incorporation of ^{3}H-Thymidine and the data presented as the mean ±SEM, (n = 7) %inhibition of proliferation (A) or %stimulation of proliferation (B) *versus* concentration (ng/ml) muIFNAR-2a.

Figure 7 is a graphical representation of the complementation of IFN activity in muIFNAR-2 -/- cells by soluble muIFNAR-2a. PHA-stimulated muIFNAR-2 -/- thymocytes were incubated with A, 100IU/ml (■) recombinant muIFNα4 or B, 100IU/ml (■) recombinant muIFNβ, with the indicated concentrations of muIFNAR-2a. Their DNA proliferation was measured by incorporation of ^{3}H-Thymidine and the data is presented as the mean ±SEM (n = 4) %inhibition of proliferation *versus* concentration (ng/ml) muIFNAR-2a. Note that murine IFNAR 2 (-/-) thymocytes are not responsive to muIFNα4 nor muIFNβ in the absence of soluble receptor.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated, in part, on the elucidation of the biological activity of the soluble IFNAR 2a molecule. The elucidation of this activity has facilitated the development of a method of regulating type I interferon functional activity in a subject.

Accordingly, one aspect of the present invention provides a method of regulating, in a subject, interferon functional activity said method comprising administering to said subject an effective amount of a soluble interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said soluble interferon receptor to couple, bind or otherwise associate with said interferon.

More particularly, the present invention provides a method of regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said soluble type I interferon receptor to couple, bind or otherwise associate with said type I interferon.

Reference herein to "type I interferon" should be read as including reference to all proteins falling within the type I interferon family of proteins and to derivatives, homologues, analogues, equivalents or mimetics thereof. This includes, by way of example, interferon-α subtypes, interferon-β, interferon-ϖ and interferon-τ (Pestka *et al.*, 1987). Preferably said type I interferon is interferon α or interferon β. It should be understood that type I interferon, the activity of which is regulated in accordance with the method of the present invention, may be either endogenously produced interferon or exogenously introduced interferon. Where it is desired to up-regulate type I interferon functional activity by presenting it via the soluble IFNAR 2a receptor, the type I interferon and IFNAR 2a may be individually administered either simultaneously or sequentially or they may be administered as a complex.

In a preferred embodiment, the present invention provides a method of regulating, in a subject, interferon α and/or interferon β functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative thereof for a time and under conditions sufficient for said soluble type I interferon receptor to couple, bind or otherwise associate with said interferon α and/or interferon β.

In another aspect the present invention is directed to a method of regulating, in a subject, interferon functional activity said method comprising administering to said subject an effective amount of a soluble interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof coupled, bound or otherwise associated with type I interferon, or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said complex to regulate interferon functional activity.

Preferably said interferon is type I interferon and even more preferably interferon α or interferon β.

The term "soluble type I interferon receptor" refers to a molecule, the ligand for which is a type I interferon molecule, and which can exist in soluble form. The soluble receptor may be either naturally occurring or may be recombinently or chemically synthesised to both bind a type I interferon molecule, such as interferon-α and/or interferon β, and to exist in soluble form. This term also encompasses all isomeric forms or precursor forms of these receptors and receptor components. This definition should also be understood to encompass fully glycosylated, partially glycosylated and unglycosylated forms of the receptor, such as are recombinantly produced by yeast and *E. coli*, for example. Reference to the soluble type I receptor molecule should be understood, to the extent that it is not otherwise stated, to include reference to derivatives, homologues, analogues, equivalents or mimetics thereof. "Derivatives", which are more fully defined below, include fragments or parts thereof. Accordingly, the receptor molecule suitable for use in the present invention may comprise part of the receptor from which it is derived, for example the isolated ligand binding region. The type I interferon receptor is known to

comprise two protein chains - IFNAR 1 together with either IFNAR 2b or 2c. Accordingly, reference to derivatives includes reference to all or part of one or more of these protein chains. It also includes reference to all isomeric forms of IFNAR proteins, and in particular IFNAR 1 and 2, such as the naturally occurring soluble form of the IFNAR 2 receptor component termed IFNAR 2a. Preferably, said soluble type I interferon receptor is an IFNAR protein and even more preferably the IFNAR 2 isoform-IFNAR 2a (Owczarek *et al.*, 1997; Lutfalla *et al.*, 1995; Novick *et al.*, 1994). Without limiting the present invention in any way, IFNAR 2a is encoded by one of the multiple mRNA transcripts transcribed from the IFNAR 2 gene. This isoform is a soluble isomeric form of the IFNAR 2 receptor component.

Although the present invention is exemplified with respect to human and murine IFNAR 2a molecules, it should be understood to extend to any soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof, regardless of its source.

In a preferred embodiment, the present invention provides a method of regulating, in a subject, interferon α and/or interferon β functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative thereof for a time and under conditions sufficient for said soluble type I interferon receptor to couple, bind or otherwise associate with said interferon α and/or interferon β.

In another aspect the present invention is directed to a method of regulating, in a subject, interferon functional activity said method comprising administering to said subject an effective amount of a soluble interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof coupled, bound or otherwise associated with type I interferon, or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said complex to regulate interferon functional activity.

Derivatives include fragments, parts, portions, chemical equivalents, mutants, homologs, mimetics from natural, synthetic or recombinant sources including fusion proteins.

Derivatives may be derived from insertion, deletion or substitution of amino acids. Amino acid insertional derivatives include amino and/or carboxylic terminal fusions as well as intrasequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Additions to amino acid sequences including fusions with other peptides, polypeptides or proteins.

The derivatives of said components include fragments having particular epitopes or parts of the entire component fused to peptides, polypeptides or other proteinaceous or non-proteinaceous molecules. For example, said components or derivative thereof may be fused to a molecule to facilitate its entry into a cell. Analogs of said components contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecules or their analogs. Derivatives of nucleic acid sequences may similarly be derived from single or multiple nucleotide substitutions, deletions and/or additions including fusion with other nucleic acid molecules. The derivatives of the nucleic acid molecules of the present invention include oligonucleotides, PCR primers, antisense molecules, molecules suitable for use in cosuppression and fusion of nucleic acid molecules.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS);

acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carboethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl

alanine and/or D-isomers of amino acids. A list of unnatural amino acid contemplated herein is shown in Table 1.

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg

D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr

D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe

carbamylmethyl)glycine		carbamylmethyl)glycine
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc	

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH).

Reference to the "functional activity" of type I interferon should be understood as a reference to any one or more of the activities which type I interferon induces, enhances or otherwise facilitates. For example, type I interferons induce antiviral, antiproliferative and immunomodulatory activities. Although often unwanted, type I interferons are also known to induce fever, malaise, nausea and leukopenia.

Type I interferons mediate their functional activities by both direct and indirect means. For example, where type I interferons bind to a cell surface receptor and thereby, for instance, inhibit the proliferation of that cell or up- or down-regulate the expression of a cell surface receptor, its activities are direct. Where type I interferons act on a cell to induce the production of other regulatory molecules, such as other cytokines, this activity is also direct. However, to the extent that these induced regulatory molecules directly or via the production of still further regulatory molecules ultimately induce the activities of, for instance, proliferation or cell surface receptor expression, the activity of interferon is indirect. The method of the present invention should be understood to extend to the regulation of both the direct and the indirect functional activity of type I interferons.

Reference to "regulating" type I interferon functional activity is a reference to up-regulating, down-regulating or otherwise modulating the functional activity of type I

interferon. It should also be understood that the modulation of any given functional activity, for example with respect to up- or down-regulation, may be partial or complete. In this regard it should be understood that said regulation may be achieved by modulating the actions of endogenously produced type I interferon, for example by blocking its cell surface receptor binding site using IFNAR 2a or by more efficiently presenting type I interferon to a cell surface receptor using IFNAR 2a. "Regulation" may also be achieved by introducing exogenously type I interferon, for example, as a complex with IFNAR 2a for the purpose of efficient presentation of the subject cytokine and the induction of type I interferon biological activities. "Regulating" should also be understood to extend to the modulation of type I interferon biological activities using agents, which have been identified via the screening method hereinafter described, which modulate the functioning molecules of the type I interferon signalling pathway. Preferably said regulation is down regulation. Down regulation of type I interferon activity is desirable where minimisation of the adverse side effects of naturally produced type I interferon or type I interferon therapy is required. These adverse side effects include, for example, inhibition of normal cell growth, fever, malaise, nausea, leukopenia and the development of disease conditions such as autoimmune disease or Aicardi-Goutières Syndrome. Adverse side effects may also occur in disease conditions such as Downs Syndrome where chromosome 21, which comprises the gene for the interferon receptor, is expressed in an extra copy.

According to this preferred embodiment, the present invention provides a method of down-regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said receptor to couple, bind or otherwise associate with said type I interferon.

Preferably, said type I interferon is interferon-α and/or interferon β and even more preferably said receptor is IFNAR 2a.

Without limiting the invention to any one theory or mode of action, down-regulation of type I interferon functional activity is achieved by inhibiting the binding of type I interferon to its cell surface receptor due to the binding of IFNAR 2a to the interferon molecule thereby blocking its cell surface receptor binding site. Although the preferred method is to down-regulate interferon functional activity, up-regulating the functional activity of type I interferon may be desired under certain circumstances. For example, where a subject is either not responding or weakly responding or responding in the wrong manner to endogenously produced or exogenously administered type I interferon.

Still without limiting the present invention to any one theory or mode of action, conversely to blocking the binding of type I interferon to its cell surface receptor, IFNAR 2a can also present its ligand (type I interferon) to the IFNAR 1 component of the type I interferon receptor and facilitate transduction of a signal into the target cell in response to the binding of the type I interferon molecule. It is thought that this is achieved due to the action of the soluble receptor in holding the interferon ligand at the cell surface IFNAR 1 receptor chain for a sufficient length of time and at a sufficiently high affinity such that signal transduction is achieved. In this regard, the inventors have demonstrated that type I interferon can be presented, by the soluble receptor, to cells including those which express the transmembrane IFNAR 1 molecule but lack the transmembrane IFNAR 2 molecule. Upon presentation of the interferon molecule, a signal is transduced into the cell and type I interferon functional activity has been effectively up-regulated. Reference to "up-regulating" type I interferon activity should therefore be understood to include the induction of a type I interferon signal in a cell via a partial cell surface interferon receptor. In particular, the receptor comprises only the IFNAR 1 molecule.

Again, without limiting the present invention to any one theory or mode of action, it is thought that the ability of IFNAR 2a to either inhibit interferon activity or up-regulate defective or non-existent interferon activity is linked to the proportions of IFNAR 1 and IFNAR 2 on the surface of a cell, being a ratio which might vary from cell to cell. Where a normal cell has equal numbers of IFNAR 1 of IFNAR 2 on the cell surface, or if IFNAR 2 is in excess, then the presence of soluble IFNAR 2a would be expected to

inhibit IFN binding to cell surface receptors and to block IFN activity. By contrast, if a cell has little or no surface IFNAR 2, or if IFNAR 1 is in excess to IFNAR 2, then the presence of soluble IFNAR 2a could bind IFN and present it to cell surface IFNAR 1 thereby complementing the actions of IFN. This provides a potential mechanism b which IFNAR 2a activity can be predicted and thereby approximately applied to a given therapeutic or prophylactic situation.

Accordingly, the present invention is also directed to a method of up regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said receptor to couple, bind or otherwise associate with said type I interferon.

Preferably, said type I interferon is interferon-α and/or interferon β and even more preferably said receptor is IFNAR 2a.

The subject soluble type I interferon receptor and type I interferon molecules which are coupled, bound or otherwise associated may be associated by any mechanism including, but not limited to, via covalent bonds, ionic bonds, hydrogen bonds, van Der Waals forces or any other bonding mechanism.

In another aspect there is provided a method of up-regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative, homologue, analogue, chemical equivalent or mimetic thereof coupled, bound or otherwise associated with type I interferon or derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to up-regulate type I interferon functional activity.

Preferably, said type I interferon is interferon α and/or interferon β and even more preferably said receptor is IFNAR 2a.

In accordance with the present invention, it is proposed that IFNAR 2a is a soluble type I interferon receptor component which is a naturally occurring isomeric form of the IFNAR 2 protein and which exhibits functional activity, in particular, the regulation of type I interferon functional activity. In addition to the regulation of type I interferon functional activity by administering IFNAR 2a or derivative, homologue, analogue, equivalent or mimetic thereof, the present invention also extends to the modulation of the expression of the IFNAR 2 gene, to produce the IFNAR 2a isoform, by administering an agent which is capable of up-regulating, down-regulating or otherwise modulating IFNAR 2 gene expression. Also contemplated, are the therapeutic and prophylactic uses of IFNAR 2a nucleic acid molecules or derivatives, homologues, analogues, equivalents or mimetics thereof or IFNAR 2a agonistic and antagonistic agents for the regulation of IFNAR 2a functional activity. Hereinafter, reference to the IFNAR 2 gene will appear in italics as "*IFNAR 2*" and reference to the expression product of the gene will appear in ordinary text. *IFNAR 2* should be understood to include reference to any nucleic acid molecule encoding IFNAR 2 or a derivative of IFNAR 2 including DNA and RNA. *IFNAR 2* should also be understood to encompass derivatives, homologue, analogue, equivalent or mimetic thereof.

The following aspects of the present invention, where reference is made to *IFNAR 2* or IFNAR 2a, should also be understood to extend to soluble type I interferon receptors as hereinbefore defined and their encoding nucleic acid molecules.

Accordingly, another aspect of the present invention provides a method of regulating type I interferon functional activity in a subject said method comprising administering to said subject an effective amount of a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and

under conditions sufficient for the expression product of said nucleic acid molecule to couple, bind or otherwise associate with said type I interferon.

Still another aspect of the present invention provides a method of regulating, in a subject, type I interferon functional activity said method comprising contacting *IFNAR 2* with an effective amount of an agent for a time and under conditions sufficient to up-regulate, down-regulate, or otherwise modulate expression of *IFNAR 2*. For example, oligonucleotides which down-regulate translation of *IFNAR 2* mRNA transcripts are envisaged. Also envisaged is the administration of a nucleic acid molecule encoding IFNAR 2a or a derivative thereof which may be introduced to effectively up-regulate IFNAR 2a expression.

Another aspect of the present invention contemplates a method of regulating activity of type I interferon in a subject, said method comprising administering to said subject an effective amount of an agent for a time and under conditions sufficient to modulate IFNAR 2a activity.

Reference to modulating the "activity" of IFNAR 2a should be understood as a reference to modulating the ability of IFNAR 2a to regulate the activity of type I interferon, for example, by modulating its capacity to bind, couple or otherwise associate with type I interferon or modulating the nature of this association.

Regulation of type I interferon activity by the administration of an agent to a mammal can be achieved by one of several techniques, including but in no way limited to introducing into said mammal a proteinaceous or non-proteinaceous molecule which:

(i) modulates expression of *IFNAR 2*;

(ii) functions as an antagonist of IFNAR 2a;

(iii) functions as an agonist of IFNAR 2a (including administration of IFNAR 2a or derivative, homologue, analogue, equivalent or mimetic thereof).

Said proteinaceous molecule may be derived from natural or recombinant sources including fusion proteins or following, for example, natural product screening. Said non-proteinaceous molecule may be, for example, a nucleic acid molecule or may be derived from natural sources, such as for example natural product screening or may be chemically synthesised. The present invention contemplates chemical analogs of IFNAR 2a capable of acting as agonists or antagonists of IFNAR 2a. Chemical agonists may not necessarily be derived from IFNAR 2a but may share certain conformational similarities. Alternatively, chemical agonists may be specifically designed to mimic certain physiochemical properties of IFNAR 2a. Antagonists may be any compound capable of blocking, inhibiting or otherwise preventing IFNAR 2a from carrying out its normal biological functions. Antagonists include monoclonal antibodies specific for IFNAR 2a, or parts of IFNAR 2a, and antisense nucleic acids which prevent transcription or translation of *IFNAR 2* genes or mRNA in mammalian cells.

Said proteinaceous or non-proteinaceous molecule may act either directly or indirectly to modulate the expression of *IFNAR 2* or the activity of IFNAR 2a. Said molecule acts directly if it associates with *IFNAR 2* or IFNAR 2a to modulate the expression or activity of *IFNAR 2* or IFNAR 2a, respectively. Said molecule acts indirectly if it associates with a molecule other than *IFNAR 2* or IFNAR 2a, which other molecule either directly or indirectly modulates the expression or activity of *IFNAR 2* or IFNAR 2a, respectively. Accordingly, the method of the present invention encompasses the regulation of *IFNAR 2* or IFNAR 2a expression or activity via the induction of a cascade of regulatory steps which lead to the regulation of *IFNAR 2* or IFNAR 2a expression or activity.

The IFNAR 2a, *IFNAR 2* or agent used may also be linked to a targeting means such as a monoclonal antibody, which provides specific delivery of the IFNAR 2a, *IFNAR 2* or agent to the target cells.

In a preferred embodiment of the present invention, the IFNAR 2a, *IFNAR 2* or agent used in the method is linked to an antibody specific for said target cells to enable specific delivery to these cells.

Administration of the IFNAR 2a, *IFNAR 2* or agent, in the form of a pharmaceutical composition, may be performed by any convenient means. IFNAR 2a, *IFNAR 2* or agent of the pharmaceutical composition are contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. The variation depends, for example, on the human or animal and the IFNAR 2a, *IFNAR 2* or agent chosen. A broad range of doses may be applicable. Considering a patient, for example, from about 0.1 mg to about 1 mg of IFNAR 2a or agent may be administered per kilogram of body weight per day. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The IFNAR 2a or agent may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intranasal, intraperitoneal, intramuscular, subcutaneous, intradermal, or suppository routes or implanting (e.g. using slow release molecules) or intrathecal. With particular reference to use of IFNAR 2a or agent, these peptides may be administered in the form of pharmaceutically acceptable nontoxic salts, such as acid addition salts or metal complexes, e.g. with zinc, iron or the like (which are considered as salts for purposes of this application). Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active ingredient is to be administered in tablet form, the tablet may contain a binder such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate.

The subject of the interferon regulation is generally an animal or bird such as but not limited to a human, primate, livestock animal (eg. sheep, cow, horse, donkey, pig), companion animal (eg. dog, cat), laboratory test animal (eg. mouse, rabbit, rat, guinea pig, hamster), captive wild animal (eg. fox, deer), caged bird (eg. parrot) and poultry

bird (eg. chicken, duck, pheasant, turkey). Preferably, the subject is a human or primate. Most preferably, the subject is a human.

The method of the present invention is useful in the treatment and prophylaxis of disease conditions including, but not limited to, auto-immune disease (eg., diabetes melitis or SLE, Aicardi-Goutières Syndrome, Downs Syndrome, graft vs host disease, diseases involving type I interferon therapy where unwanted side effects are observed or diseases involving unwanted interferon production due either to the subject's immune response which is naturally induced as a result of the disease condition or the treatment schedule itself. Any unwanted type I interferon effects, whether occurring naturally due to the immune response or due to therapeutic treatment, are referred to herein as "unwanted type I interferon functional activity".

Accordingly, in another aspect there is provided a method for the treatment or prophylaxis of a disease condition characterised by unwanted type I interferon functional activity in a subject said method comprising administering to said subject an effective amount of one of more of:

(i) a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(ii) a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, equivalent, homologue, analogue or mimetic thereof;

(iii) an agent capable of modulating the activity of a soluble type I interferon receptor; or

(iv) an agent capable of modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor

sufficient to down-regulate the functional activity of said type I interferon.

Said down regulation may be either the partial or complete inhibition of type I interferon functional activity.

Preferably said type I interferon is interferon α and/or interferon β and even more preferably said soluble type I interferon receptor is IFNAR 2a.

Under certain circumstances, though, it may be desirable to up-regulate the functional activity of type I interferon. For example, where an individual is not able to adequately respond to naturally produced type I interferon or administered type I interferon or where the individual exhibits only a weak response or where immunostimulation is otherwise required. These responses are referred to herein as an "inadequate type I interferon response". In this regard, administration of soluble IFNAR 2a to up-regulate the interferon response, that is to facilitate or augment the functional activity of type I interferon, is useful.

Accordingly, in another aspect of the present invention there is provided a method for the treatment or prophylaxis of a disease condition characterised by an inadequate type I interferon response in a subject said method comprising administering to a subject an effective amount of one or more of:

(i) a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(ii) a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, equivalent, homologue, analogue or mimetic thereof;

(iii) an agent capable of modulating the activity of a soluble type I interferon receptor; or

(iv) an agent capable of modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor

sufficient to up-regulate the functional activity of said type I interferon.

Preferably said type I interferon is interferon α and/or interferon β and even more preferably said soluble interferon receptor is IFNAR 2a.

Routes of administration include but are not limited to intravenously, intraperitioneal, subcutaneously, intracranial, intradermal, intramuscular, intraocular, intrathecal, intracerebrally, intranasally, infusion, orally, rectally, *via* iv drip, patch and implant. Intravenous routes are particularly preferred.

An "effective amount" means an amount necessary at least partly to attain the desired response, or to prevent or to delay the onset or inhibit progression or halt altogether, the onset or progression of a particular condition being treated. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the degree of protection desired, the nature of formulation the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Reference herein to "treatment" and "prophylaxis" is to be considered in its broadest context. The term "treatment" does not necessarily imply that a subject is treated until total recovery. Similarly, "prophylaxis" does not necessarily mean that the subject will not eventually contract a disease condition. Accordingly, treatment and prophylaxis include amelioration of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylaxis" may be considered as reducing the severity of onset of a particular condition. "Treatment" may also reduce the severity of an existing condition or the frequency of acute attacks (for example, reducing the severity of initial onset).

In accordance with these methods, the molecules administered in accordance with the present invention may be coadministered with one or more other compounds or

molecules. By "coadministered" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours or days between the administration of the two types of molecules, These molecules may be administered in any order.

The present invention further extends to the use of the subject soluble type I receptors, nucleic acid molecules encoding said receptors or agents which regulate the activity or expression of said receptors or encoding nucleic acid molecules in the manufacture of a medicament for the treatment of disease conditions characterized either by unwanted type I interferon functional activity or inadequate type I interferon responses.

Yet another aspect of the present invention provides an agent useful for regulating interferon functional activity comprising a soluble interferon receptor, nucleic acid molecules encoding said receptors or agents which regulate the activity or expression of said receptors or encoding nucleic acid molecules as hereinbefore defined.

Another aspect of the present invention relates to a method of treating a subject said method comprising administering to said subject an effective amount of an agent for a time and under conditions sufficient to modulate the expression of *IFNAR 2* or sufficient to modulate the activity of IFNAR 2a wherein said modulation results in modulation of type I interferon functional activity.

In another aspect the present invention relates to a method of treating a subject said method comprising administering to said subject an effective amount of IFNAR 2a or *IFNAR 2* or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient to regulate type I interferon functional activity.

Yet another aspect of the present invention relates to the use of an agent capable of modulating the expression of *IFNAR 2* or modulating the activity of IFNAR 2a in the manufacture of a medicament for the regulation of type I functional activity.

A further aspect of the present invention relates to the use of IFNAR 2a or *IFNAR 2* or derivative, homologue, analogue, equivalent or mimetic thereof in the manufacture of a medicament for the regulation of type I interferon functional activity.

Still yet another aspect of the present invention relates to agents for use in modulating *IFNAR 2* expression or IFNAR 2a activity wherein said regulation results in modulation of type I interferon functional activity.

Another aspect of the present invention relates to IFNAR 2a or *IFNAR 2* or derivative, homologue, analogue, equivalent or mimetic thereof for use in modulating type I interferon functional activity.

Another aspect of the present invention provides a pharmaceutical composition for use in regulating interferon functional activity comprising a soluble interferon receptor as hereinbefore defined, *IFNAR 2* or derivative, homologue, analogue, equivalent or mimetic thereof or an agent capable of modulating *IFNAR 2* expression or IFNAR 2a activity together with any one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to as the active ingredients.

Compositions suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. They must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought

about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by, for example, filter sterilization or sterilization by other appropriate means. Dispersions are also contemplated and these may be prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, a preferred method of preparation includes vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution.

When the active ingredients are suitably protected, they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1ng and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil

of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The present invention also extends to forms suitable for topical application such as creams, lotions and gels. In such forms, the active ingredients may need to be modified to permit penetration of the surface barrier.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the

particular therapeutic effect to be achieved and (b) the limitations inherent in the art of compounding such an active material.

Effective amounts of the composition contemplated by the present invention will vary depending on the severity of the pain and the health and age of the recipient. In general terms, effective amounts may vary from 0.01 ng/kg body weight to about 100 mg/kg body weight. Alternative amounts include for about 0.1 ng/kg body weight about 100 mg/kg body weight or from 1.0 ng/kg body weight to about 80 mg/kg body weight.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of expressing a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof. The vector may, for example, be a viral vector.

Without limiting the invention in any way, the expression of IFNAR 2a is thought to be regulated independently of the expression of the transmembrane IFNAR 2 component. It is found in serum and other biological fluids and considerable variation between subjects is observed with respect to the ratio of soluble receptor to transmembrane receptor. Since populations of individuals who either cannot respond or weakly respond to type I interferon (ie. the type I interferon has little or no functional activity) have been observed, it is thought that this poor response may be due to high levels of circulating soluble IFNAR 2a which binds the interferon thereby blocking its capacity to bind to interferon receptors. For example, individuals suffering from hepatitis B and C usually undergo interferon α treatment. Interferon-α is administered to reduce viral replication. In westerners, 70% of patients are observed to respond to interferon-α treatment. However, among Asians, the response rate drops to less than 30%. Individuals who cannot respond or weakly respond to type I interferon are referred to herein as "type I interferon non-responders". The identification of subjects who are type I interferon non-responders would facilitate the design and application of alternative therapeutic and prophylactic protocols to obviate the consequence of an inability to adequately respond to type I interferon.

Accordingly, another aspect of the present invention relates to a method of determining type I interferon non-responsiveness in a subject said method comprising screening for the level of a soluble type I interferon receptor or a derivative, homologue, analogue, equivalent or mimetic thereof in a body fluid of said subject wherein the level of said soluble receptor or derivative, homologue, analogue, equivalent or mimetic thereof relative to the normal level of said soluble receptor or derivative, homologue, analogue, equivalent or mimetic thereof is indicative of type I interferon non-responsiveness.

Reference to "body fluid" should be understood to include fluids derived from the body of said subject such as, but not limited to, urine, blood (including all blood derived components, for example, serum and plasma), tears, mucus and fluids which have been introduced into the body of said subject and subsequently removed. Preferably, the body fluid is serum.

The method of the present invention is predicated on the correlation of levels of soluble type I interferon receptor in the serum of subjects with a normal level of said soluble receptor. The "normal level" is the level of soluble receptor in the serum of a subject who is not exhibiting a disease condition. Said "normal level" may be a discrete level or a range of levels.

Preferably, said body fluid is serum and even more preferably said soluble receptor is IFNAR 2a.

The levels of IFNAR 2a can be detected by any suitable method which would be well known to those skilled in the art. For example, in one particular preferred method the target molecules in the sample are exposed to a specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with an antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second

antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of target-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody-target complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-target-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of target which was present in the sample.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by

emission of the light at a characteristic colour visually detectable with any suitable detection system. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first molecule complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the molecule of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

The present invention also contemplates genetic assays such as involving PCR analysis to detect soluble type I interferon receptor mRNA levels.

Another aspect of the present invention provides a kit for detecting soluble type I interferon receptor said kit comprising in compartmental form a first compartment adapted to contain a type I receptor specific immunointeractive molecule and a second compartment adapted to contain reagents useful for visualising said immunointeractive molecule. Further compartments may also be included, for example to receive a biological sample such as a serum sample.

Yet another aspect of the present invention is directed to antibodies to soluble type I interferon receptor molecules. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies or may be specifically raised to said molecules. The present invention extends to recombinant and synthetic antibodies and to antibody hybrids.

Specific antibodies can be used to screen for serum levels of soluble type I interferon receptor molecules. Alternatively, they can be used to regulate the functional activity of said soluble receptor molecules by inhibiting their capacity to bind type I interferon. This may be of particular use where an individual overproduces soluble type I interferon receptors.

As detailed above, the inventors have determined that interferon complexed to a soluble receptor, such as IFNAR 2a, can result in the transmission of an intracellular signal via a receptor comprising only the IFNAR 1 intracellular component and not the IFNAR 2 intracellular component. Without limiting the invention to any one theory or mode of action, the inventors have shown that in the absence of the intracellular domain of IFNAR-2, the soluble IFNAR 2a-interferon-IFNAR 1 complex transduces fewer signals than a normal cell. Since it is these signals which lead to the induction of the various biological effects which type I interferon is associated with (for example, anti-viral actions, inhibition of cell growth, activation of an immune cell), the soluble IFNAR 2a-interferon signalling complex may induce the expression of a specific group of genes thereby leading to the induction of specific biological responses.

Accordingly, analysis of the signalling induced by a soluble IFNAR 2a-interferon complex in a particular cell type provides the unique opportunity to "link" the induction of expression of specific genes with particular aspects of the type I interferon induced biological responses. This provides the opportunity to screen for agents which modulate either the signalling activity of the IFNAR 2a-interferon complex itself or which modulate the functional activity of molecules, such as genes, which function down-stream from the initial interferon signal. These down-stream molecules thereby become themselves potential therapeutic or prophylactic targets for the purpose of providing a mechanism by which one or more of the functional activities induced by type I interferon can be modulated.

Accordingly, another aspect of the present invention provides a method for detecting an agent capable of modulating the functional activity of type I interferon, said method comprising contacting a cell or extract thereof, which cell or extract thereof contains IFNAR 1 and/or IFNAR 2, or its functional equivalent or derivative thereof, with an IFNAR 2a-interferon complex and a putative agent and detecting an altered expression phenotype associated with said type I interferon.

It should be understood that said putative agent may function as an agonist or an antagonist of said interferon or of a molecule which functions as part of the downstream type I interferon signalling pathway.

Reference to detecting an "altered expression phenotype associated with said type I interferon" should be understood as the detection of cellular changes associated with modulation of the activity of interferon. These may be detectable for example as intracellular changes or changes observable extracellularly. For example, this includes, but is not limited to, detecting changes in expression product levels or to detecting changes in reporter molecule expression. Alternatively, this screening system may be established to detect changes in the expression or activity of downstream molecules following interferon stimulation. For example, detecting changes in mRNA or expression product levels of cytokines which are induced following interferon stimulation of a cell.

The IFNAR 2a-interferon complex may be administered together with or sequentially to the putative agent of interest. Reference to "modulating the functional activity of type I interferon" should be understood to have the same meaning as detailed earlier. That is, it is a reference to the modulation of one or more of the functional activities which type I interferon is known to induce. In this regard, where an agent is identified which up-regulates the activity of a molecule which acts down stream during interferon signalling, the subsequent up regulation of the activity of this molecule by said agent, in the absence of any interferon stimulation, should be understood to fall within the scope of the phrase "modulating the functional activity of interferon".

In another aspect, the present invention should also be understood to extend to secondary screening methods which are designed to detect agents which regulate the activity of interferon induced signals, which screening methods do not require the use of IFNAR 2a and/or type I interferon during the testing procedure. Specifically, where the screening method hereinbefore defined has identified type I interferon related signalling pathways, subsequent testing for modulatory agents, to the extent that it is directed to those

identified signals would not necessarily require the on going use of a screening method which relies on the presence and/or activity of type I interferon or IFNAR 2a during the screen.

In one preferred embodiment, IFNAR 2 -/- cells are treated with interferon plus IFNAR 2a to identify a subset of signals that are responsible for an interferon induced effect. This knowledge (of specific signals and/or specific effect) can then be used to implement a secondary screen for small molecules that induce the newly identified signal, and thereby a specific biological/therapeutic effect, potentially independently of the present of interferon and/or the soluble receptor.

In another aspect, the present invention relates to modulatory agents detected in accordance with the screening method herein disclosed.

Still another aspect of the present invention relates to the use of said modulatory agents to modulate type I interferon functional activity in accordance with the method of the present invention.

Further features of the present invention are more fully described in the following non-limiting examples. It is to be understood, however, that the following description is included solely for the purpose of exemplifying the present invention.

EXAMPLE 1
NORTHERN BLOTTING

Poly(A)$^+$ mRNA from cell lines, BMM or organs were prepared by a modification of a published method (Gonda *et al.*, 1982). Poly(A)$^+$ mRNA (3μg) was denatured with formamide, fractionated on a 1% agarose gel containing 0.67% formaldehyde and electrophoresed in buffer containing 20mM MOPS (pH 7.0), 5mM sodium acetate and 1mM EDTA, then transferred to Hybond-C Extra nitrocellulose (Amersham) membranes in 20X SSC according to the manufacturers' instructions. The membranes were hybridised with a ^{32}P-labelled murine IFNAR-2 cDNA, then stripped and rehybridised with a labelled 1.1-kb glyceraldehyde phosphate dehydrogenase cDNA probe as described previously (Owczarek *et al.*, 1997). Quantitative analysis was performed using a Fuji BAS 1000 phosphorimage analyser.

EXAMPLE 2
PROTEIN EXPRESSION

A BamHI/EcoRI-digested fragment encoding the muIFNAR-2a cDNA was cloned into a pGEX-2T vector for expression in *E. coli*. Expression of recombinant muIFNAR-2a in an overnight culture of *E. coli* was induced with 50μM Isopropyl β-D-Thiogalactopyranoside (IPTG) for 4 hours at 30°C. Lysis of the cell pellet by sonication on ice in 1% Triton X-100, 0.1mM Phenylmethylsulfonyl Fluoride using a 4710 Ultrasonics Homogeniser was followed by centrifugation and collection of the supernatant. To the supernatant was added 50% (w/v) Glutathione Sepharose (Pharmacia) to bind the muIFNAR-2a fusion protein, and after 4 hours of incubation at 4°C, the sepharose beads were washed extensively to remove non-specific proteins. To cleave the muIFNAR-2a protein from the sepharose beads, 15IU Thrombin (Boehringer Mannheim) was added for 2 hours at 37°C, and 50% (w/v) Benzamidine Sepharose (Pharmacia) was then incubated with the supernatant for 2 hours at 4°C to remove any thrombin from the protein preparation. The concentration of muIFNAR-2a was determined using known standards.

A cDNA fragment encoding the murine IFNAR-2 extracellular domain (ECD) with a human myc-9E10 epitope (Evan *et al.*, 1985) at the 5′ end and a His-tag at the 3′ end was obtained by PCR using a published technique called Splicing by Overlap Extension (Ho *et al.*, 1989). The PCR product was digested with SnaBI and XbaI and directionally cloned into a SnaBI/AvrII-digested commercial pPIC9 vector (Invitrogen) to ensure secreted expression of the protein in *Pichia pastoris*. Sequence analysis confirmed that the construct was in the correct reading frame. The plasmid was digested with SalI, then transformed into a *P. pastoris* GS115 yeast strain according to the manufacturers' recommendations (Invitrogen). Selection of yeast clones correctly expressing the muIFNAR-2 ECD protein was performed by Western blot using a mouse anti-myc monoclonal antibody, and optimisation of methanol concentrations (1.5%) and expression duration (48 hours) were followed by large-scale protein production. Yeast culture supernatant containing recombinant muIFNAR-2 ECD was then transferred to dialysis tubing and buffer-exchanged into 20mM Tris, 100mM NaCl (pH 8.0) for 24 hours at 4°C to allow for purification by metal affinity chromatography. This purification was performed under native conditions using TALON cobalt resin according to the manufacturers' recommendations (Clontech). The purified muIFNAR-2 ECD from this process was concentrated 40-fold using Centricon-10 columns according to the manufacturers' recommendations (Amicon) and the protein concentration determined against known standards.

EXAMPLE 3
ANTIBODY PRODUCTION

New Zealand White rabbits were immunised three times with approximately 150μg of purified muIFNAR-2 ECD secreted from *P. pastoris*. Following the immunisation protocol, the rabbits were bled and their serum collected. An antibody titre of > 10000 was confirmed by ELISA.

EXAMPLE 4

WESTERN BLOTTING

SDS-PAGE was performed according to the method of Cleveland *et al.* (1977), using the vertical Biorad electrophoresis apparatus. Proteins separated by SDS-PAGE were transferred onto Hybond-C extra presoaked in 25mM Tris, 192mM Glycine, 0.1% SDS, 20% Methanol using a Semiphor transfer apparatus (Hoeffer) at 100mA for 1-2 hours. All reactions were carried out at room temperature (RT). Following transfer, membranes were incubated in 1% BSA (Sigma)/0.1% Tween-20 (ICN) in PBS for 1 hour to block non-specific binding sites, then for another hour upon addition of a 1:5000 dilution of the primary rabbit anti-muIFNAR-2 ECD antibody in BSA/Tween. Membranes were washed extensively in BSA/Tween, then incubated with a 1:2000 dilution of the secondary HRP-conjugated goat anti-rabbit Ig antibody. Membranes were washed in 0.1% Tween/PBS as above, visualised by autoradiography using enhanced chemiluminescence (ECL) reagents (Supersignal; Pierce). Prestained molecular weight markers (Biorad) were used to estimate protein sizes.

Where a recombinant muIFNAR-2a competitor was used, the primary antibody and competitor were pre-incubated together for 1 hour at 37°C before being added to the membrane for 1 hour at RT. Membrane washing, incubation with the secondary antibody and visualisation by autoradiography using ECL reagents are as above.

EXAMPLE 5

TRANSIENT TRANSFECTIONS

Murine IFNAR-2a and -2c cDNA were directionally cloned into pEF-BOS for expression in Simian COS7 cells, which were grown to confluency in 175cm^2 flasks (Falcon) using DMEM (10% FCS, 1%P/S) as the growth medium. The cells were washed with PBS, trypsinised, resuspended in 50ml PBS and centrifuged at 1500rpm for 5' before being resuspended in 800μl/flask PBS and placed on ice. To a 0.4cm electroporation cuvette (Biorad) was added 20μg of the cDNA to be used and 800μl of the resuspended cells.

The mixture was electroporated on a Biorad Genepulser II, resuspended in 1ml DMEM, layered over 1ml FCS and centrifuged at 1500rpm for 5'. The cells were resuspended in 5ml media and added to 175cm^2 flasks previously coated in 10ml 1% gelatin (in PBS) at room temperature for 30'. Another 40ml of fresh media was added, and the cells incubated at 37°C (5% CO_2) for 3 days. At this stage, the conditioned media was collected, centrifuged at 1500rpm for 5' to remove debris, and the supernatant concentrated 10-fold using Centricon-10 columns (Amicon).

EXAMPLE 6

STABLE TRANSFECTIONS AND CLONAL SELECTION

A murine L929 cell line was grown to confluency in RPMI (10% FCS, 1% P/S), trypsinised, resuspended in PBS to a concentration of 1 x 10^7 cells/ml and stably transfected with 10μg of a NotI-digested p250ASLucNeo cDNA (Holland *et al.*, 1997) by electroporation. Cells were selected with 600μg/ml G418. After 2 weeks of selection, 24 positive clones were picked and tested for luciferase inducibility with 100IU/ml recombinant muIFNα1 in RPMI. Following 7 hours of incubation at 37°C (5% CO_2) with IFN, the media was removed, the cells incubated in lysis buffer (Promega) for 15' at room temperature and 20μl supernatant analysed on a Lumat LB9501 Luminometer (Berthold) using the Luciferase assay system (Promega) reagents. The clone giving the greatest induction of luciferase activity upon IFN treatment was chosen for the reporter assay and maintained in G418.

EXAMPLE 7

REPORTER ASSAY

The stably transfected L cell line was grown to confluency in RPMI (10% FCS, 1% P/S), trypsinised, and reseeded into 24-well plates at a concentration of 1 x 10^5 cells/well. After 24 hours incubation at 37°C (5% CO_2), the media was removed. To each well of adherent cells was added 10IU/ml, 1IU/ml or no muIFNα1/muIFNβ (toray) pre-incubated at 37°C for 1 hour with 100ng/ml, 10ng/ml, 1ng/ml or no recombinant

muIFNAR-2a (*E. coli*), or muIFNAR-2a heat-inactivated at 95°C for 10′ to a total of 1ml in RPMI. This was performed in duplicate wells. The plates were incubated at 37°C (5% CO_2) for 7 hours, then the cells in each well analysed for luciferase activity with the luciferase assay system and Luminometer as above.

EXAMPLE 8
THYMIDINE INCORPORATIONS

Thymuses from CF1 or C57Bl/6 age-matched mice, or muIFNAR-2 -/- mice were removed and their cells dispersed in PBS through a fine mesh sieve. The cells were centrifuged at 1750rpm for 5′, resuspended in 10ml RPMI (5% FCS, 1% P/S, 2.5 x 10^{-5}M β-Mercaptoethanol) and counted using a Neubauer Haemocytometer. To each well of a 96-well flat-bottomed plate (Falcon) was added 5 x 10^5 cells in media, followed by 40μg/ml Phytohaemagglutinin (PHA; Sigma) and recombinant IFN/IFNAR-2a if required. Plates were incubated at 37°C, 5% CO_2 for 30 hours, then for another 18 hours after the addition of 0.5μCi (1μCi/μl) ^{3}H-Thymidine (ICN) to each well. The thymocytes were harvested in a Beckman 96-well micromate cell harvester onto glass fibre disks (Packard), which were placed into separate scintillation vials with 1ml/vial of Scintillation fluid (National Diagnostics) and counted in a 1900TR Liquid Scintillation Analyzer (Packard).

EXAMPLE 9
ADULT MURINE IFNAR-2 EXPRESSION

Extensive Northern blot analyses of poly(A)$^+$ mRNA obtained from a wide panel of murine tissues and cell lines using a full-length murine IFNAR-2 cDNA probe, show two major transcripts of approximately 1.5-kb and 3.8-kb present (Fig. 1A). In most tissues, it is apparent that the intensity of the 1.5-kb transcript encoding the soluble receptor is more intense than the 3.8-kb transcript encoding the transmembrane isoform. Interestingly, murine cell lines 3T3, L929 and primary fibroblasts also express muIFNAR-2 transcripts. This expression pattern is confirmed by quantitative analysis of

the Northern blots (Fig. 1B), whereby the ratio of muIFNAR-2a : 2c in most tissues is 3.5-5.5 : 1. A few tissues, such as the small intestine and liver, show expression of the 1.5-kb transcript up to 15 times greater than the 3.8-kb transcript. By contrast, in the thymus, spleen and bone marrow-derived macrophages (Figs. 1A and 1B), the ratio of the transcripts is equal to or less than 1 : 1. Thus, importantly it has been shown that the expression of the transcript encoding the soluble receptor muIFNAR-2a, is regulated independently of the transmembrane muIFNAR-2c isoform, and varies up to 20-fold between organs.

EXAMPLE 10

DEVELOPMENTAL MURINE IFNAR-2 EXPRESSION

Northern blot analyses of poly(A)$^+$ mRNA obtained from a number of murine tissues at several stages of embryonic development using a full-length murine IFNAR-2 cDNA probe, show the same 1.5-kb and 3.8-kb transcripts as observed in adult tissues in all tissues tested (Fig. 2A). It is notable that the intensity of the two transcripts are more similar in the embryonic tissues than in the adult tissues. A histogram of the ratios of the soluble to transmembrane transcript intensities in developmental murine tissues (Fig. 2B) show an increase with development. At E10 and E12, the 2a:2c ratio is less than one. The trend in organs such as brain, liver and intestine is to increase the 2a:2c ratio during development, but the most dramatic change in the ratio occurs between the neonatal and adult stages, especially in the liver and intestine. By contrast, a few organs maintain a low ratio throughout development until adulthood e.g. lung and thymus. This data suggests that up-regulation of the soluble receptor encoded by the 1.5-kb transcript occurs during murine development and further implies functional importance for the soluble receptor.

EXAMPLE 11

EXPRESSION OF SOLUBLE MURINE IFNAR-2a PROTEIN

It was important to demonstrate that the cDNA encoding muIFNAR-2a could produce protein. Recombinant muIFNAR-2a was produced in *E.coli* and migrated at approximately 29kDa upon SDS-PAGE when probed with a rabbit anti-muIFNAR-2 extracellular domain (ECD) primary antibody by Western blot (Fig. 3A , panel 1). This confirms the predicted size of 28.9kDa derived from the cDNA sequence. Recombinant muIFNAR-2 extracellular domain produced in the yeast *P. pastoris* migrated at approximately 42kDa when probed with the above antibody (Fig. 3A, panel 2), suggesting approximately 13kDa of post-translational modification (glycosylation). Similarly, a transient transfection of the muIFNAR-2a cDNA into COS7 cells produced a protein present in the conditioned media migrating at approximately 45kDa (Fig. 3A, panel 3). This protein was not present in the condition media of COS7 cells transiently tranfected with either muIFNAR-2c or vector cDNA only (Fig. 3A, panels 4 and 5), indicating that the protein is not produced by proteolytic cleavage of muIFNAR-2c at the cell surface, but from muIFNAR-2a mRNA derived by alternative splicing of the *IFNAR-2* gene. Evidence of the specificity of the polyclonal antibody used above is shown in Fig. 3B, where recombinant muIFNAR-2a (*E.coli*) is able to compete in a dose-dependent manner with the expressed muIFNAR-2a in COS7 cell conditioned media for anti-muIFNAR-2 ECD antibody binding. The band of approximately 80-85kDa is specific and is seen after protein storage. The 65kDa bands seen in most lanes are not competed and so are non-specific.

EXAMPLE 12

SOLUBLE muIFNAR-2a IN SERUM

When probed with the anti-muIFNAR-2 ECD antibody, a doublet of approximately 35kDa was seen in the serum of normal mice following SDS-PAGE (Fig. 4A). This is the same size as muIFNAR-2a expressed in COS7 cells (Fig. 3A, panel 3). Expression appears abundant, as the soluble receptor can be detected in up to a one hundred-fold

dilution of murine serum. Expression cannot be detected in the serum of mice with a null mutation of the *IFNAR-2* gene (Fig. 4A). Furthermore, we demonstrate that recombinant muIFNAR-2a is able to inhibit binding of the soluble receptor present in murine serum to antibody in a dose-dependent manner (Fig. 4B). These data indicate that the bands seen in wild-type murine serum represent the soluble receptor.

Soluble receptor is also detectable by Western blot in the urine, peritoneal fluid and saliva of mice (Fig. 4C, panels 1, 2 and 4 respectively). The soluble receptor detected in murine urine and peritoneal fluid (Fig. 4C, panels 1 and 2) migrate at similar rates to that detected in serum (Fig. 3E, panel 3), but the level of expression in these fluids appear low compared to serum levels. The soluble receptor detected in murine saliva (Fig. 4C, panel 4) migrates at approximately 85-90kDa, possibly indicating a dimeric form of the receptor; however this was unable to be reduced, even with 0.1M DTT as the reducing agent. Soluble receptor can also be detected in the conditioned media of the L929 and 3T3 cell lines (Fig. 4D, panels 2 and 3), but the levels of expression are very low relative to that of urine (Fig. 4D, panel 1). These data demonstrate that the soluble receptor protein is present in abundance in murine fluids, particularly serum.

EXAMPLE 13

BIOLOGICAL ACTIVITY OF muIFNAR-2A ON IFN-INDUCED GENE EXPRESSION

In order to determine the biological function of the soluble receptor, its effects on a murine L929 fibroblast cell line stably transfected with a construct containing the promoter of the IFN-inducible gene 2',5'-OAS linked to the luciferase gene were examined. Fig 5A shows the recombinant murine IFNα1 is able to act in a dose dependent manner to induce the 2',5'-OAS promoter as measured by induction of luciferase activity. The addition of recombinant muIFNAR-2a (*E.coli*) is shown to be able to inhibit this inducation of luciferase activity by IFNα1 in a dose-dependent manner, with significant inhibition occurring with as little as 10ng/ml (Fig. 5A). muIFNAR-2a is also able to inhibit the luciferase activity of cells where no exogenous

IFNα1 has been added, suggesting that the soluble muIFNAR-2a can inhibit the endogenous IFN responsible for the basal level of luciferase. The broad inhibitory action of the soluble receptor is confirmed by its ability to inhibit murine IFNβ-induced gene expression (Fig. 5B). Recombinant muIFNAR-2 ECD produced in the yeast *P.pastoris* had the same effect as soluble receptor produced in *E.coli*. Viable cell counts were significantly altered by the soluble receptor, indicating that the reduction was not due to a cytotoxic effect. Furthermore, receptor heat-inactivated at 95°C for 5 minutes had no significant effect on the luciferase activity of the L cells, indicating that it is the muIFNAR-2a causing the inhibition. Taken together, these data indicate an antagonistic function for the soluble receptor by preventing signal transduction caused by binding of IFN to cell surface IFNAR-1 and IFNAR-2.

EXAMPLE 14

FUNCTION OF muIFNAR-2A ON THE ANTIPROLIFERATIVE EFFECTS OF IFN

Having determined a potential antagonistic function for the soluble receptor by an *in vitro* reporter assay using the L cell line, primary murine thymocytes mitogenically stimulated with PHA were tested for activity of muIFNAR-2a on the antiproliferative effects of murine IFN. Fig. 6A shows that recombinant murine IFNα4 is able to significantly inhibit the proliferation of PHA-stimulated thymocytes with as little as 101U/ml. When recombinant muIFNAR-2a is added, a significant inhibition of IFNα4's antiproliferative actions is noted at 100ng/ml (Fig. 6A); lower concentrations had no significant effect. Heat inactivated soluble receptor had no significant effect on the PHA-stimulated thymocytes.

Upon addition of soluble receptor to PHA-stimulated thymocytes where no exogenous IFN had been added, a dose-dependent stimulation of proliferation is shown (Fig. 6B) with significant effects noted at 10ng/ml and 100ng/ml muIFNAR-2a. Taken together, these data further indicate the antagonistic role of muIFNAR-2a in cellular proliferation,

and suggest that IFN exerts a basal level of growth inhibition which muIFNAR-2a can inhibit to induce proliferation.

EXAMPLE 15

COMPLEMENTATION OF THE IFN RESPONSE IN IFNAR-2-/- MICE

PHA-stimulated thymocytes of mice with a null mutation in the *IFNAR-2* gene are unable to show significant dose-dependent antiproliferative responses to muIFNα4 or muIFNβ Upon addition of recombinant muIFNAR-2a to 100IU/ml muIFNα4 (Fig. 7A) and muIFNβ (Fig. 7B), this antiproliferative response can be complemented in a dose-dependent manner. The inhibition by 100IU/ml muIFNα4 + 100ng/ml muIFNAR-2a is 50% of the maximum inhibition of proliferation by 100IU/ml muIFNα4 on normal cells. This result indicates that soluble muIFNAR-2a can form a complex with IFN and the IFNAR-1 chain of the type I IFN receptor to elicit an antiproliferative response without the requirement of the intracellular domain of the IFNAR-2c chain. This further indicates that the IFNAR-1 chain is the main signaling chain of the receptor. Heat inactivated muIFNAR-2a elicited no significant inhibition of PHA-induced proliferation between 10ng/ml and 100ng/ml.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within its spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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CLAIMS:

1. A method of regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for said soluble interferon receptor to couple, bind or otherwise associate with said type I interferon.

2. The method according to claim 1 wherein said soluble type I interferon receptor is IFNAR 2a.

3. The method according to claim 1 or 2 wherein said type I interferon is interferon α or interferon β.

4. The method according to claim 3 wherein said regulation is down-regulation.

5. The method according to claim 4 wherein said functional activity is inhibition of cell growth, fever, malaise, nausea, leucopenia or the development or progression of autoimmune disease or Aicardi-Goutières Syndrome.

6. The method according to claim 3 wherein said regulation is up-regulation.

7. The method according to claim 6 wherein said functional activity is antiviral activity, antiproliferative activity or immunostimulatory activity.

8. A method of regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of a soluble type I interferon receptor coupled, bound or otherwise associated with type I interferon, or derivative, homologue, analogue, equivalent or mimetic thereof for

a time and under conditions sufficient for said complex to regulate type I interferon functional activity.

9. The method according to claim 8 wherein said soluble type I interferon receptor is IFNAR 2a.

10. The method according to claim 8 or 9 wherein said type I interferon is interferon α or interferon β.

11. The method according to claim 10 wherein said regulation is up-regulation.

12. The method according to claim 11 wherein said functional activity is antiviral activity, antiproliferative activity or immunostimulatory activity.

13. A method of regulating, in a subject, type I interferon functional activity said method comprising administering to said subject a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof for a time and under conditions sufficient for the expression product of said nucleic acid molecule to couple, bind or otherwise associate with said type I interferon.

14. The method according to claim 13 wherein said soluble type I interferon receptor is IFNAR 2a.

15. The method according to claim 13 or 14 wherein said type I interferon is interferon α or interferon β.

16. The method according to claim 15 wherein said regulation is down-regulation.

17. The method according to claim 16 wherein said functional activity is inhibition of cell growth, fever, malaise, nausea, leucopenia or the development or progression of autoimmune disease or Aicardi-Goutières Syndrome.

18. The method according to claim 15 wherein said regulation is up-regulation.

19. The method according to claim 18 wherein said functional activity is antiviral activity, antiproliferative activity or immunostimulatory activity.

20. A method of regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of an agent for a time and under conditions sufficient to modulate the activity of a soluble type I interferon receptor.

21. The method according to claim 20 wherein said soluble type I interferon receptor is IFNAR 2a.

22. The method according to claim 20 or 21 wherein said type I interferon is interferon α or interferon β.

23. A method of regulating, in a subject, type I interferon functional activity said method comprising administering to said subject an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a nucleic acid molecule encoding a soluble type I interferon receptor or a derivative, homologue, analogue, equivalent or mimetic thereof.

24. The method according to claim 23 wherein said soluble type I interferon receptor is IFNAR 2a.

25. The method according to claim 23 or 24 wherein said type I interferon is interferon α or interferon β.

26. A method for the treatment and/or prophylaxis of a disease condition characterised by an unwanted type I interferon functional activity in a subject said method comprising administering to said subject an effective amount of at least one of:

(i) a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(ii) a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(iii) an agent capable of modulating the activity of a soluble type I interferon receptor or derivative; or

(iv) an agent capable of modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof

for a time and under conditions sufficient to down-regulate the functional activity of said type I interferon.

27. The method according to claim 26 wherein said soluble type I interferon receptor is IFNAR 2a.

28. The method according to claim 26 or 27 wherein said type I interferon is interferon α or interferon β.

29. The method according to any one of claims 26-28 wherein said disease condition is an autoimmune disease condition, graft vs host disease or Aicardi-Goutières.

30. The method according to claim 29 wherein said autoimmune disease condition is diabetes melitis or SLE.

31. A method for the treatment and/or prophylaxis of a disease condition characterised by an inadequate type I interferon response in a subject said method comprising administering to said subject an effective amount of at least one of:

(i) a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(ii) a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(iii) an agent capable of modulating the activity of a soluble type I interferon receptor or derivative; or

(iv) an agent capable of modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof

for a time and under conditions sufficient to up-regulate the functional activity of said type I interferon.

32. The method according to claim 31 wherein said soluble type I interferon receptor is IFNAR 2a.

33. The method according to claim 31 or 32 wherein said type I interferon is interferon α or interferon β.

34. The method according to any one of claims 31-33 wherein said subject expresses insufficient levels of endogenously produced type I interferon.

35. A method of treating a subject said method comprising administering to said subject an effective amount of at least one of:

(i) a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(ii) a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(iii) an agent capable of modulating the activity of a soluble type I interferon receptor or derivative; or

(iv) an agent capable of modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof

for a time and under conditions to modulate type I interferon functional activity.

36. The method according to claim 35 wherein said soluble type I interferon receptor is IFNAR 2a.

37. The method according to claim 35 or 36 wherein said type I interferon is interferon α or interferon β.

38. The method according to claim 37 wherein said regulation is down-regulation.

39. The method according to claim 38 wherein said functional activity is inhibition of cell growth, fever, malaise, nausea, leucopenia or the development or progression of autoimmune disease or Aicardi-Goutières Syndrome.

40. The method according to claim 37 wherein said regulation is up-regulation.

41. The method according to claim 40 wherein said functional activity is antiviral activity, antiproliferative activity or immunostimulatory activity.

42. Use of a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof or a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof in the manufacture of a medicament for the regulation of type I interferon functional activity.

43. Use of an agent capable of either modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof or modulating the activity of a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof in the manufacture of a medicament for the regulation of type I interferon functional activity.

44. Use according to claim 42 or 43 wherein said soluble type I interferon receptor is IFNAR 2a.

45. Use according to claim 44 wherein said type I interferon is interferon α or interferon β.

46. An agent useful for regulating type I interferon functional activity said agent comprising one of:

(i) a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(ii) a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(iii) an agent capable of modulating the activity of a soluble type I interferon receptor or derivative; or

(iv) an agent capable of modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof

47. A pharmaceutical composition for use in regulating type I interferon functional activity said composition comprising one or more:

(i) a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(ii) a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof;

(iii) an agent capable of modulating the activity of a soluble type I interferon receptor or derivative; or

(iv) an agent capable of modulating the expression of a nucleic acid molecule encoding a soluble type I interferon receptor or derivative, homologue, analogue, equivalent or mimetic thereof

together with any one or more pharmaceutically acceptable carriers and/or diluents.

48. A method of determining type I interferon non-responsiveness in a subject, said method comprising screening for the level of a soluble type I interferon receptor or a derivative, homologue, analogue, equivalent or mimetic thereof in a body fluid of said subject wherein the level of said soluble receptor relative to the normal level of said soluble receptor is indicative of type I interferon non-responsiveness.

49. A kit for detecting soluble type I interferon receptor in a biological sample, said kit comprising in compartmental form a first compartment adapted to contain a type I receptor specific immunointeractive molecule and a second compartment adapted to contain reagents useful for visualising said immunointeractive molecule.

50. A method for detecting an agent capable of modulating the functional activity of type I interferon, said method comprising contacting a cell or extract thereof, which cell or extract thereof contains IFNAR 1 and/or IFNAR 2, or its functional equivalent, derivative, homologue, analogue or mimetic thereof, with an IFNAR 2a-interferon complex and a putative agent and detecting an altered expression phenotype associated with said type 1 interferon.

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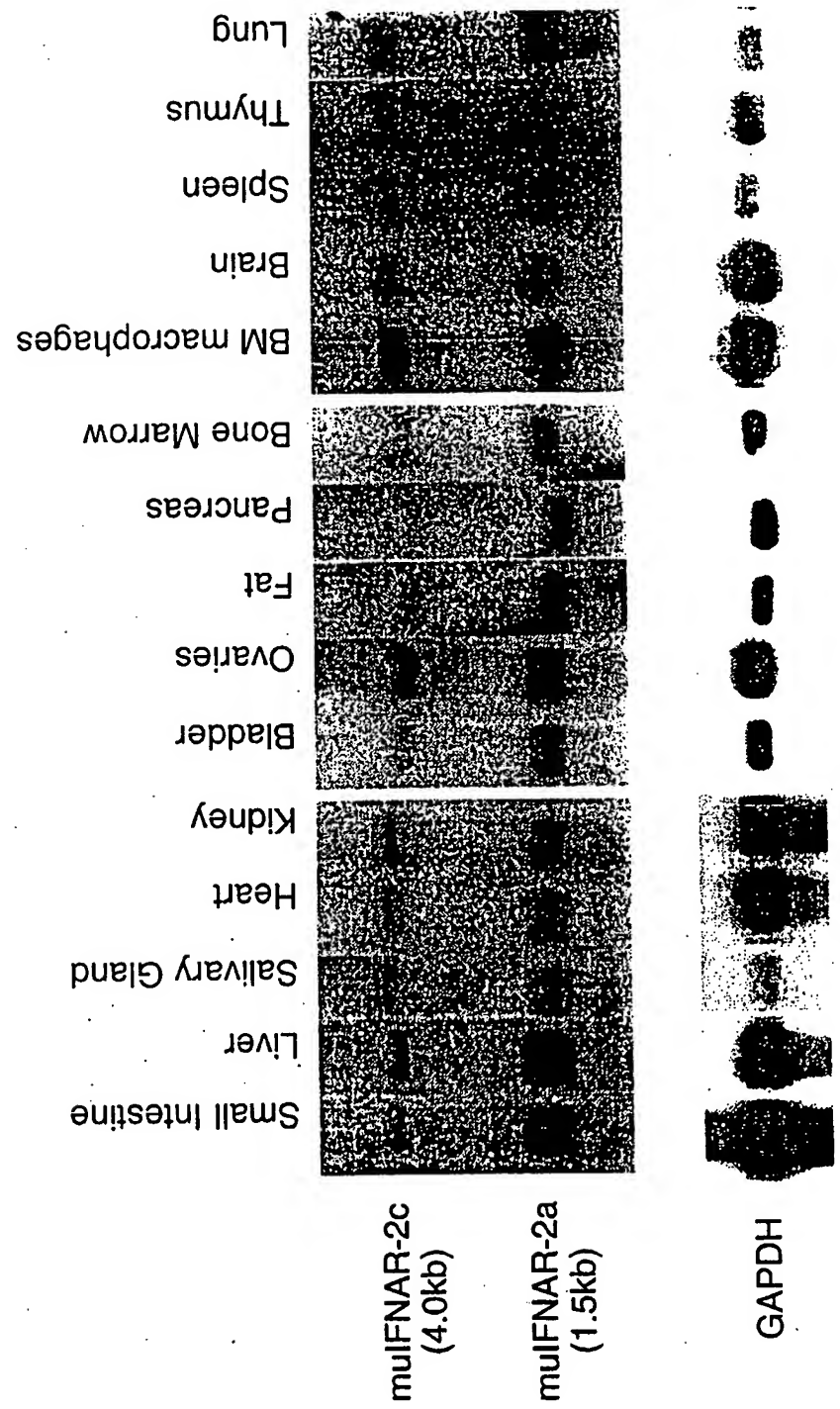

Figure 1a

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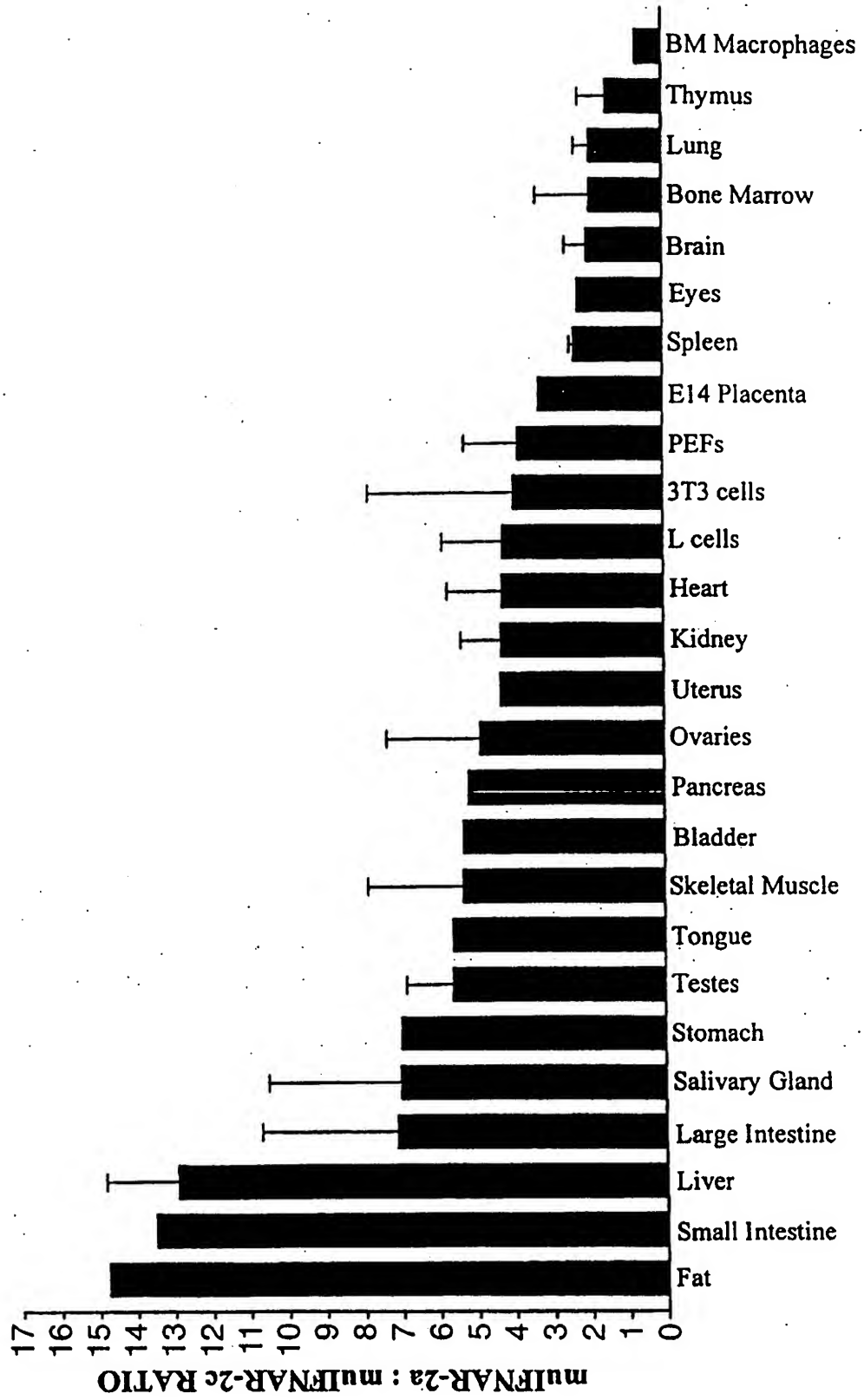

Figure 1b

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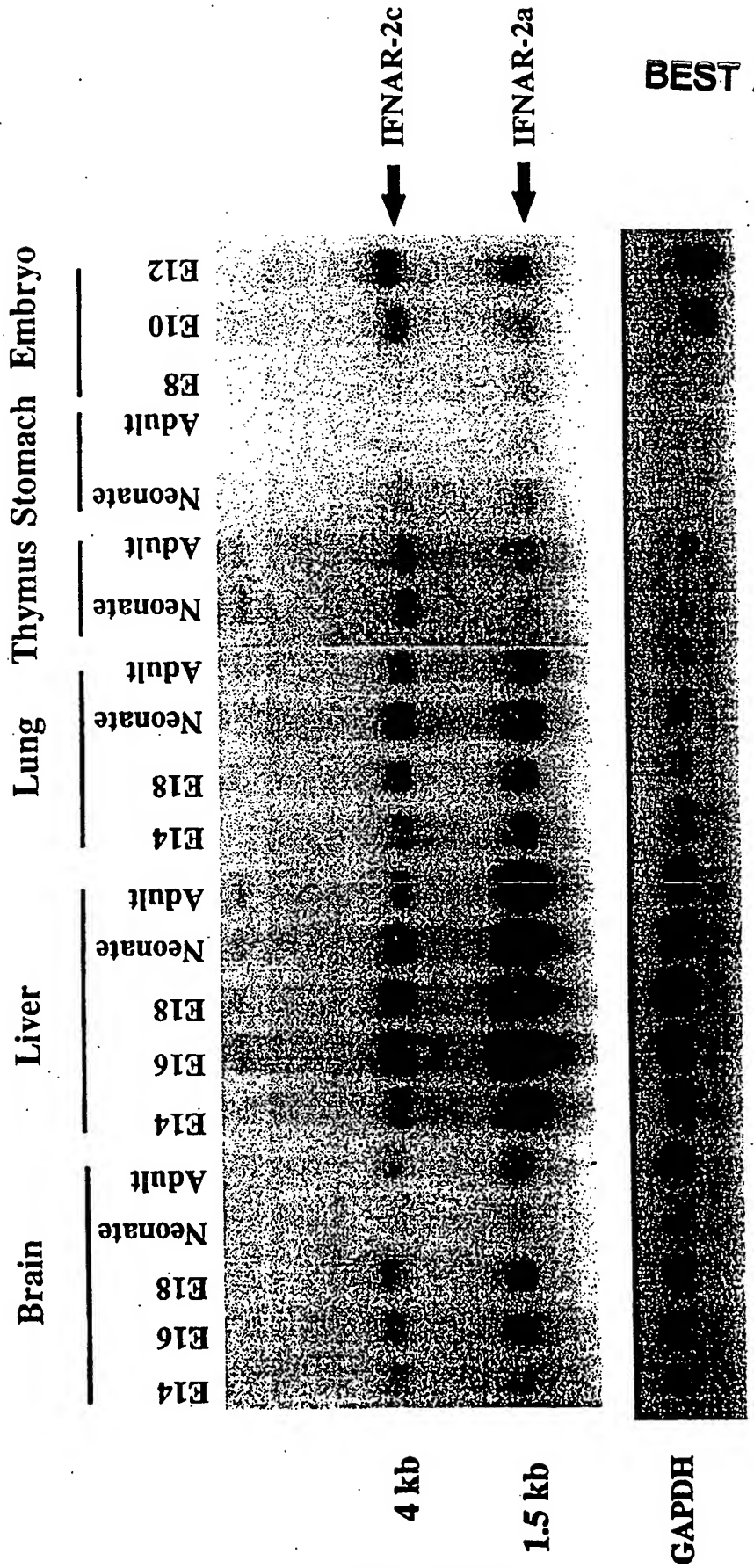

Figure 2a

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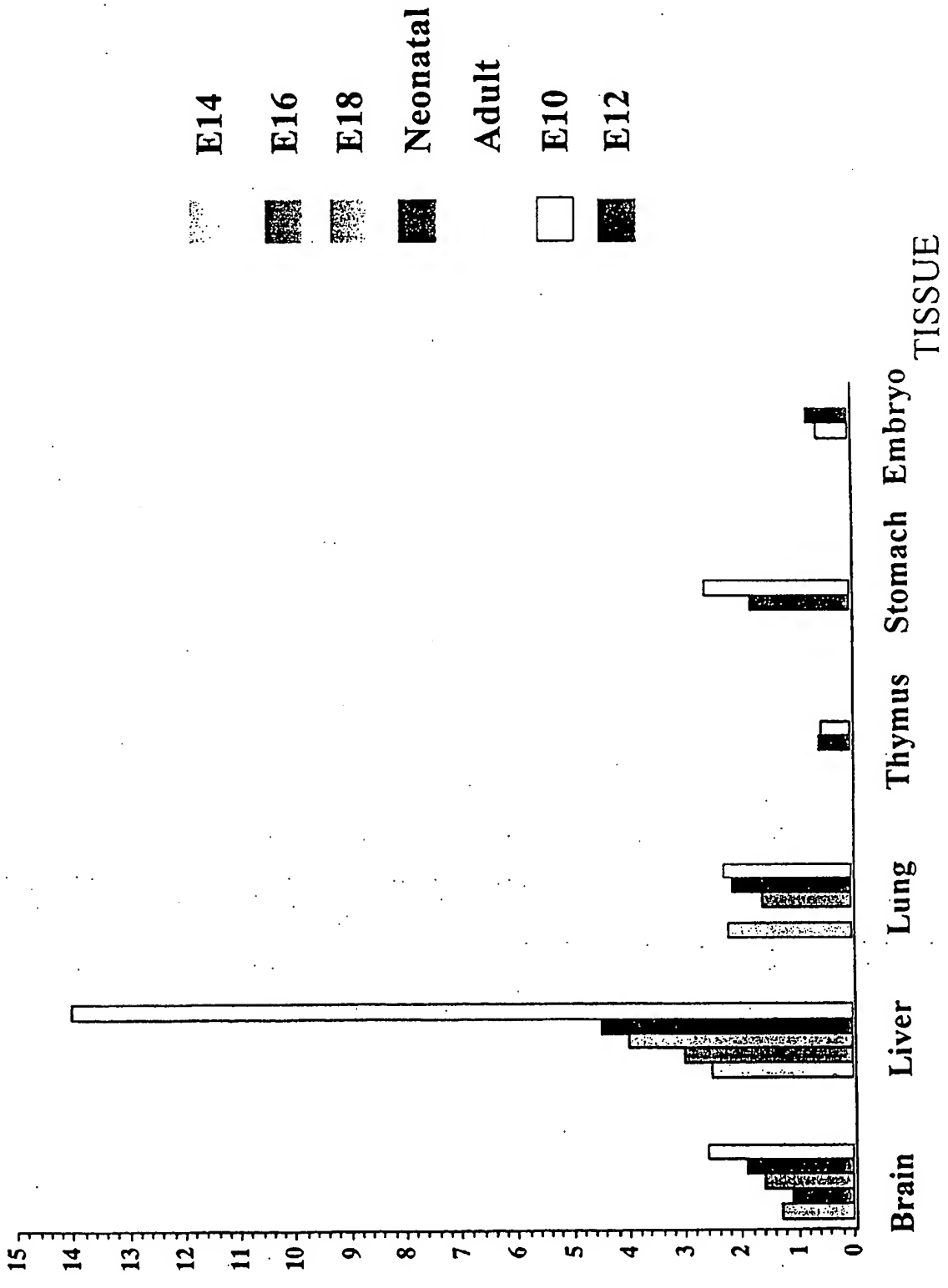

Figure 2b

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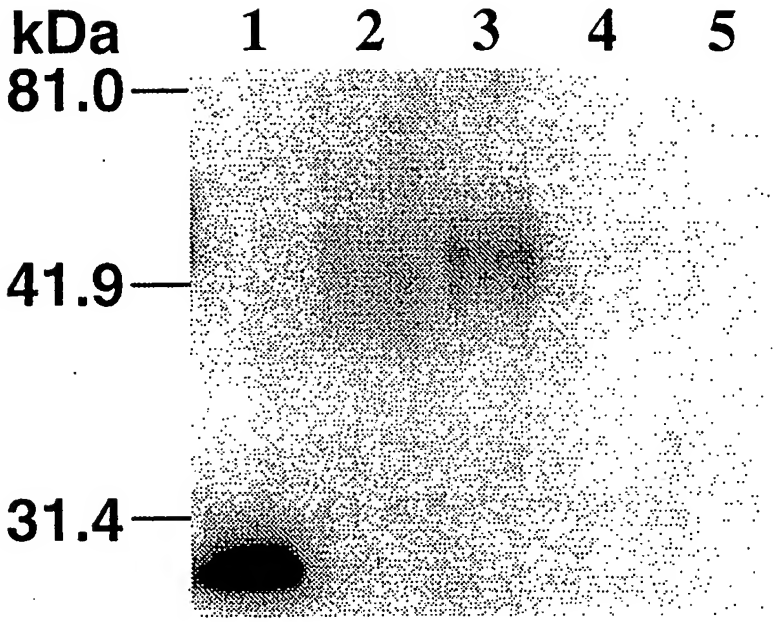

Figure 3a

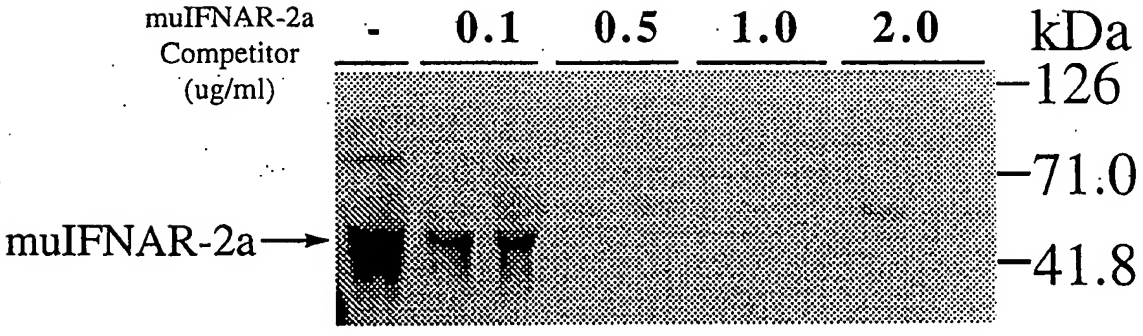

Figure 3b

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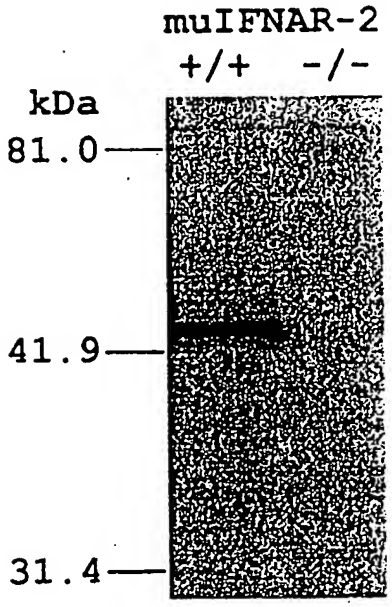

Figure 4a

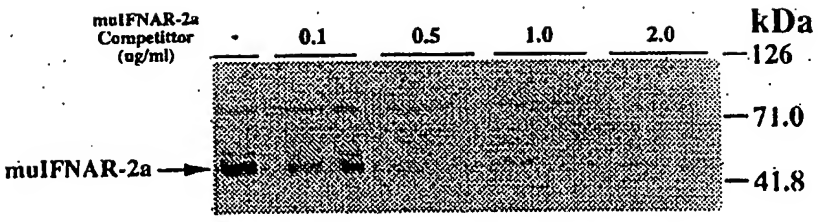

Figure 4b

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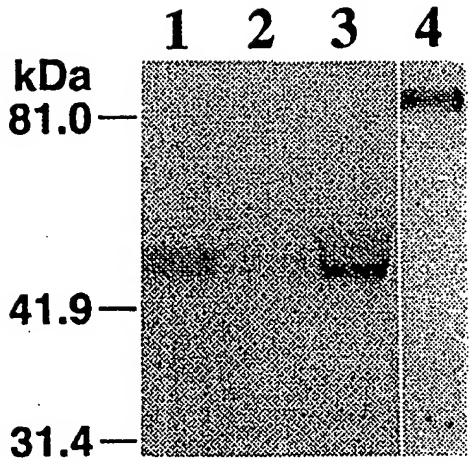

Figure 4c

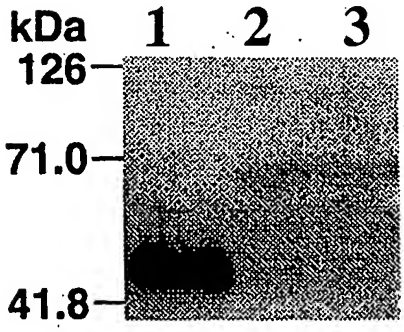

Figure 4d

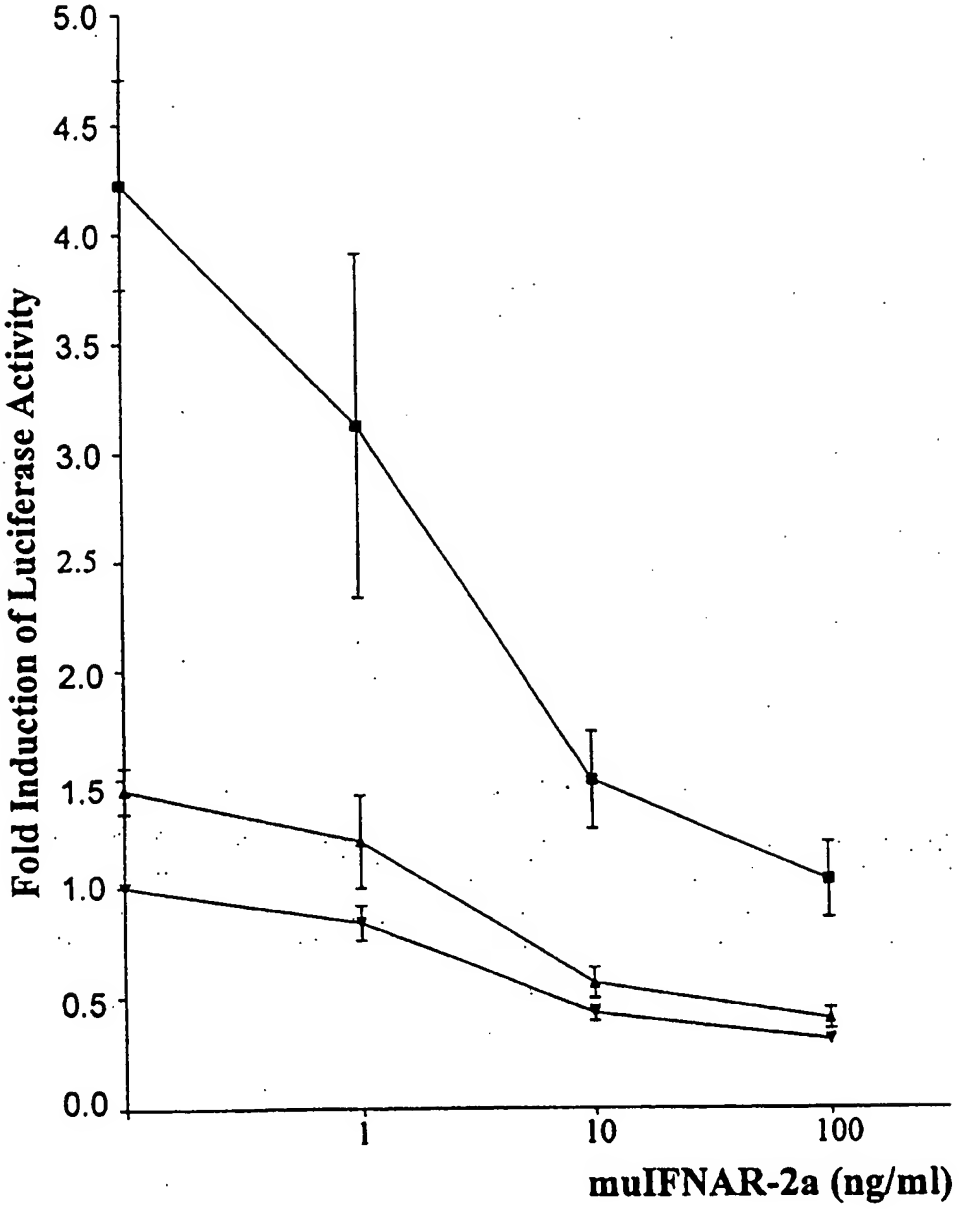
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Fold Induction of Luciferase Activity
1
10
100
muIFNAR-2a (ng/ml)

Figure 5a

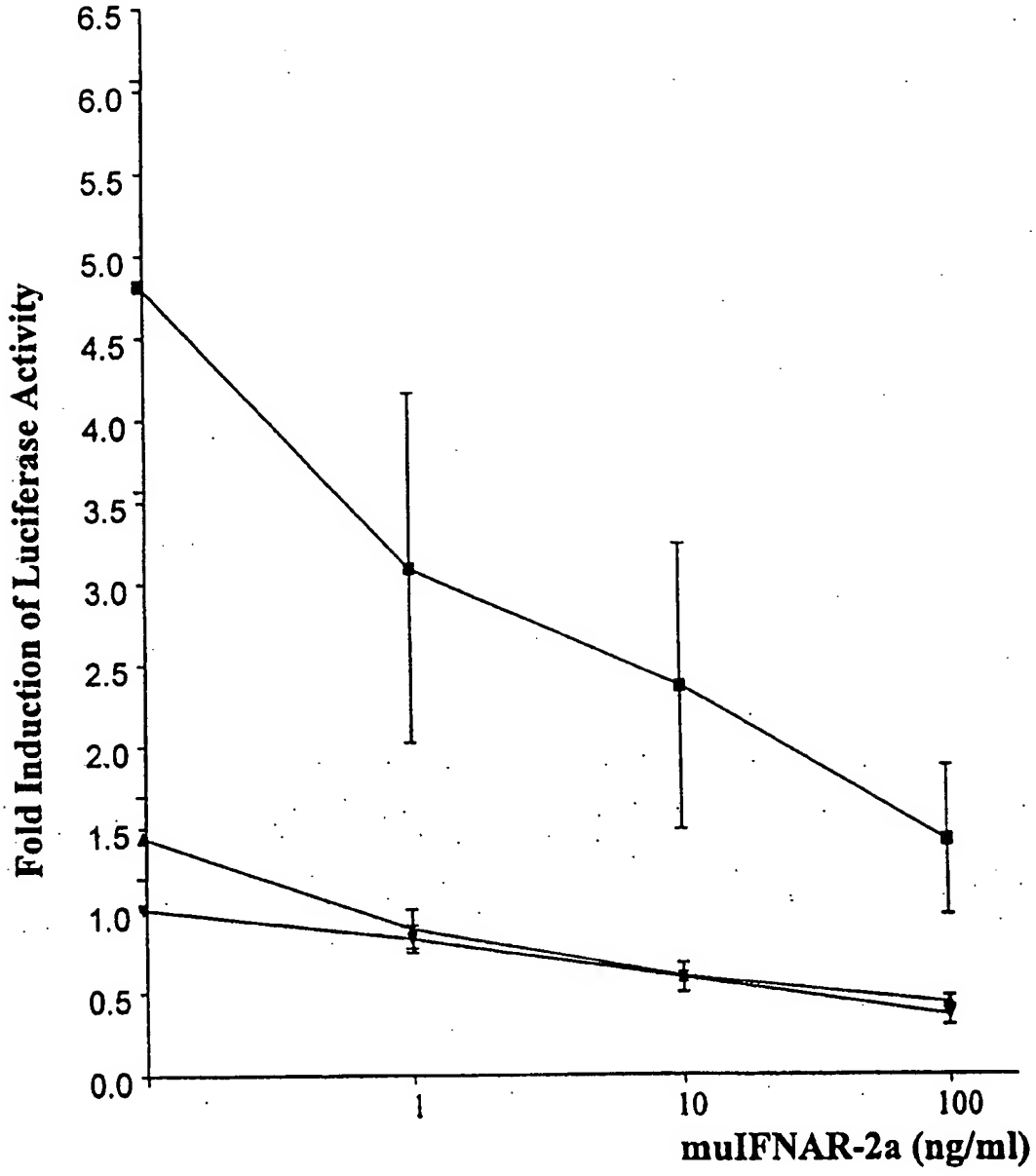
Fold Induction of Luciferase Activity
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
10
100
muIFNAR-2a (ng/ml)

Figure 5b

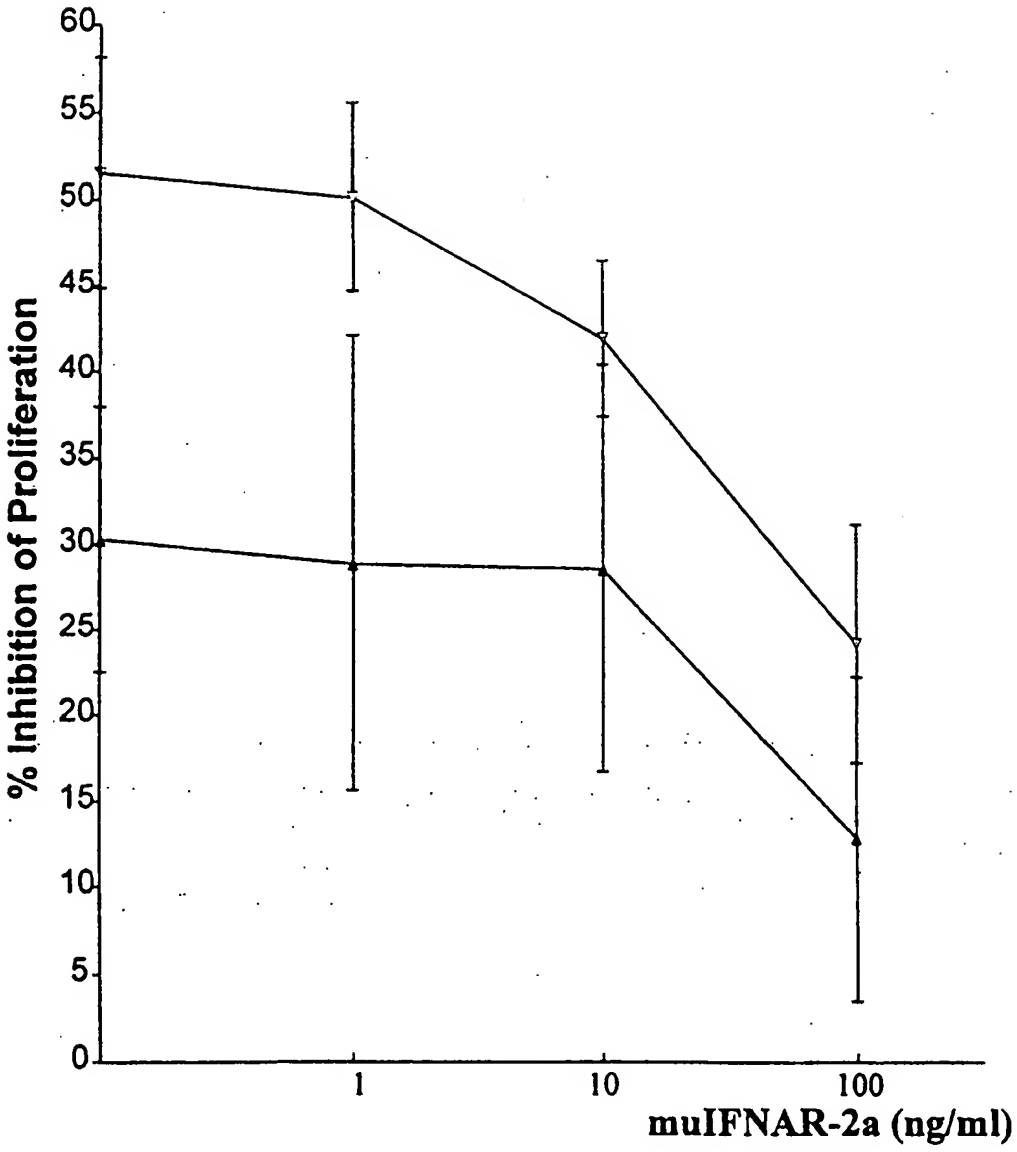
% Inhibition of Proliferation
60
55
50
45
40
35
30
25
20
15
10
5
0
1
10
100
muIFNAR-2a (ng/ml)

Figure 6a

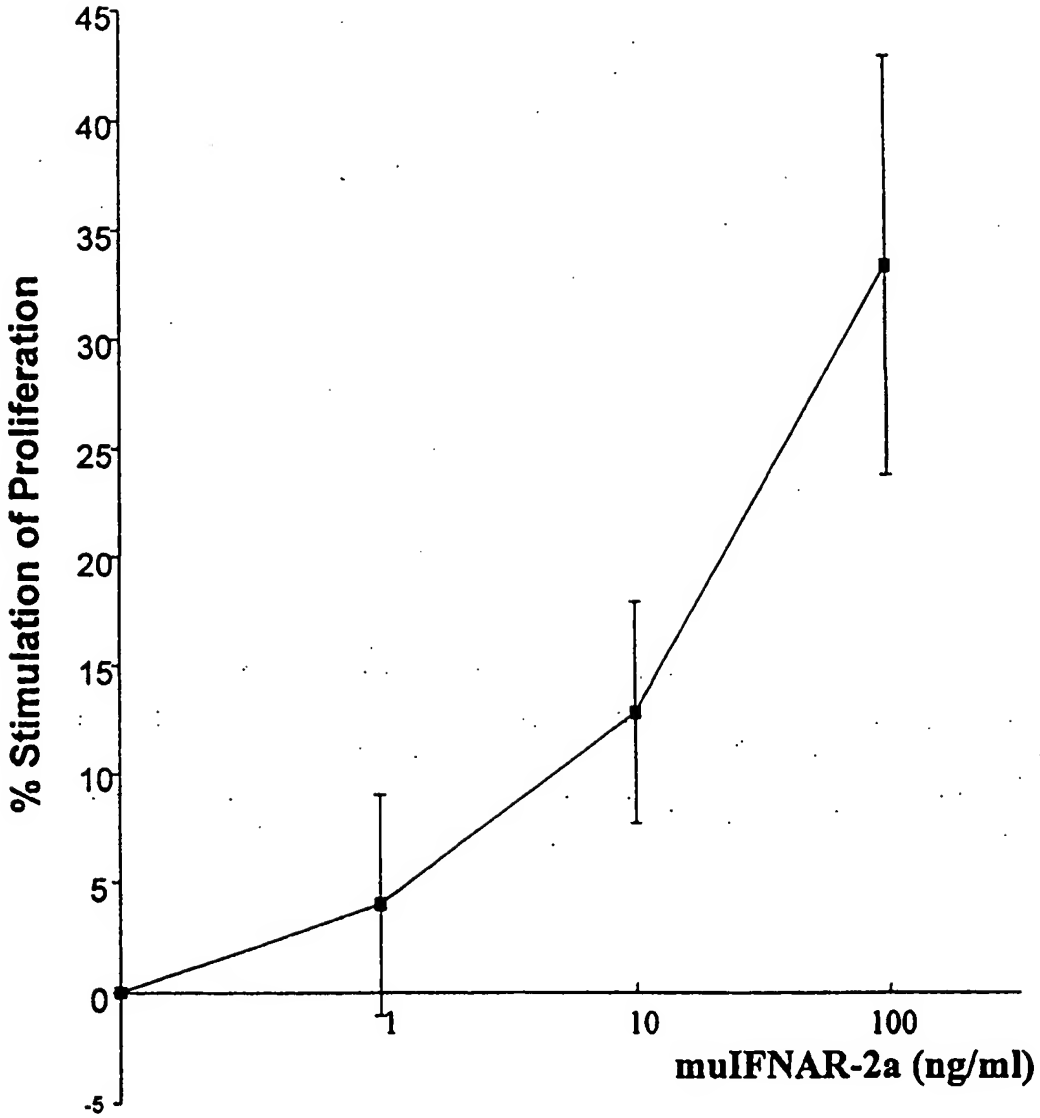
45
40
35
30
25
20
15
10
5
0
-5
% Stimulation of Proliferation
1
10
100
muIFNAR-2a (ng/ml)

Figure 6b

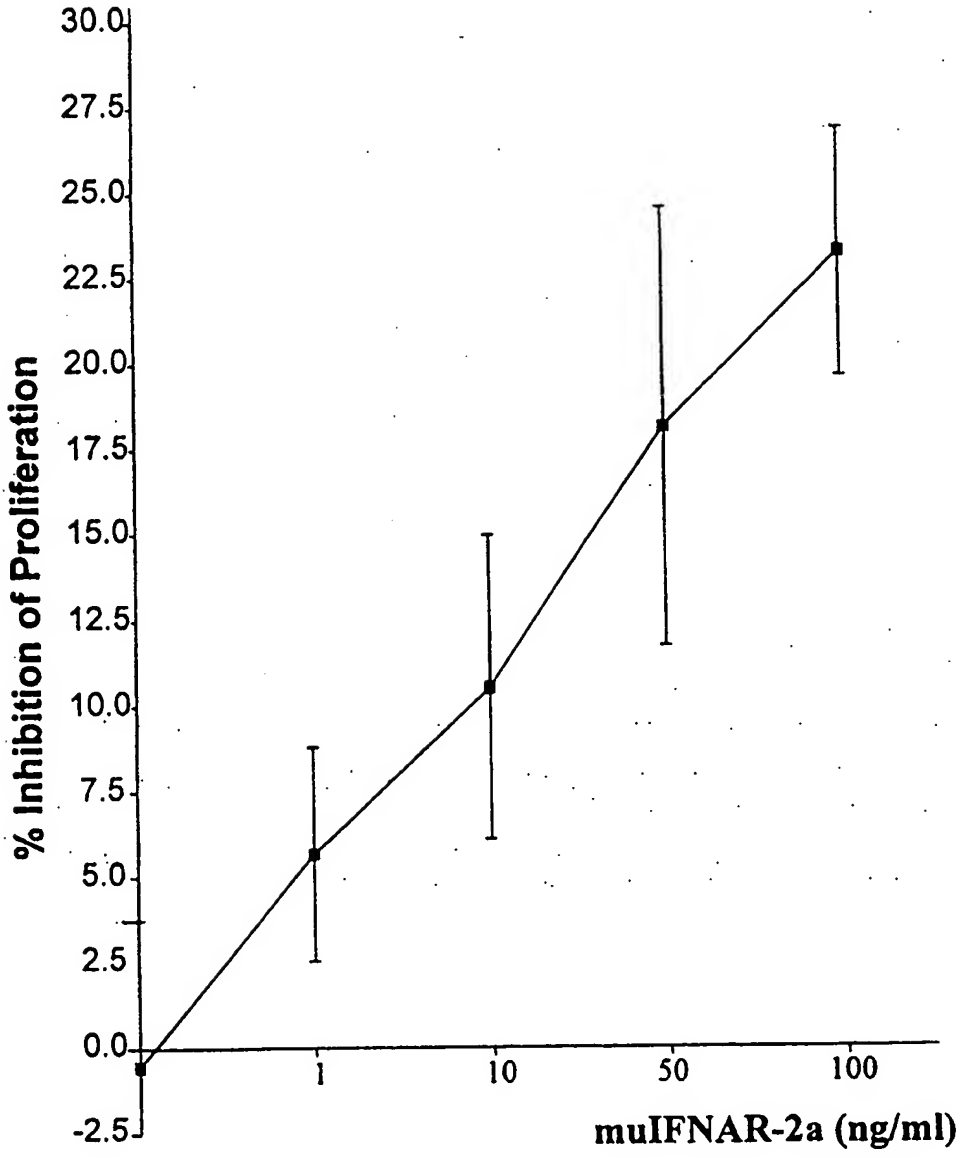
30.0
27.5
25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
-2.5
% Inhibition of Proliferation
1
10
50
100
muIFNAR-2a (ng/ml)

Figure 7a

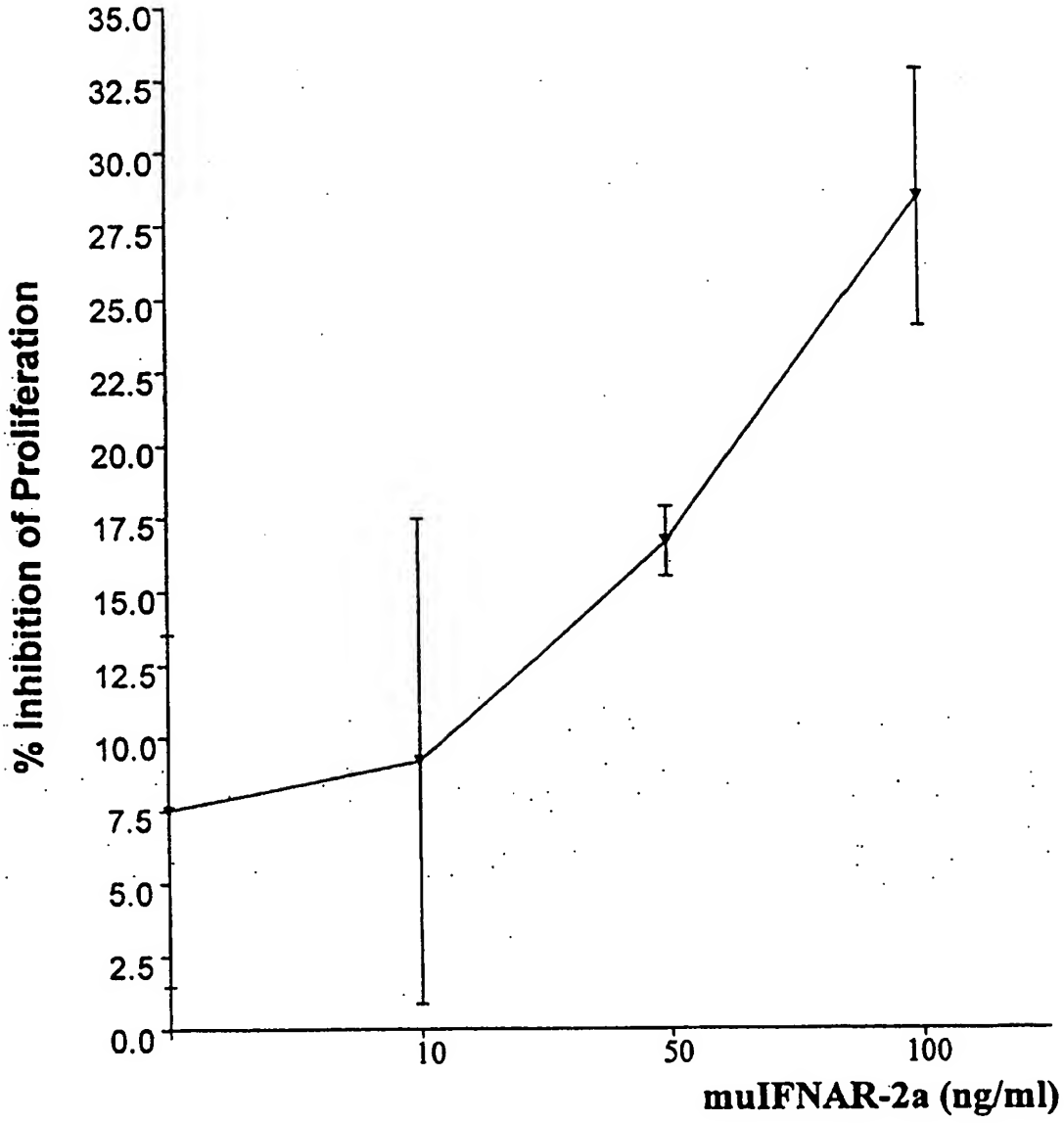
35.0
32.5
30.0
27.5
25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
% Inhibition of Proliferation
10
50
100
muIFNAR-2a (ng/ml)

Figure 7b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU99/00915

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 38/21, G01N, 33/68, 33/566

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A61K, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT
CAPLUS } INFAR 2a or soluble INFAR
MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Novick, D., et al. Cell, Vol 77 "The Human Interferon α/β Receptor: Characterization and Molecular - Cloning" pp 391 - 400, May 6, 1994.	1, 8, 13, 20, 26, 47 and 49
P,X	WO 99/32141 A (Applied Research Systems Ars Holding N.V.) 1 July 1999	8, 42
X	Owczarek, C.M., et al. The Journal of Biological Chemistry, Vol 272, No. 38, "Cloning and Characterization of soluble and Transmembrane Isoforms... IFNAR 2", pp23865 - 23870. 19 Sept 1997	1 - 47 and 49

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 December 1999	05 JAN 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU99/00915

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Hirata, R.D.C., et al. Biotechnology Techniques. Vol 11. No. 5. "Optimized production of the soluble human interferon & receptor (IFNAR) expressed in E. coli." pp 301 - 305. May 1997	1 - 47 and 49
X	AU 75977/94 (684103) B (YEDA RESEARCH & DEVELOPMENT CO LTD) 11 May 1995	1 - 47 and 49
X	Abramovich. C., et al. FEBS letters vol 338 "Identification of mRNA's encoding two different soluble forms of the human interferon a - receptor". pp 295 - 300. Feb 1994	1 - 47 and 49

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU99/00915

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	99/32141	AU	19269/99				
AU	75977/94	CA	2134030	EP	649903	EP	679717
		JP	7236488	US	5643749	ZA	9408345

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